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SWIMMING SPEED, TAIL BEAT FREQUENCY, TAIL BEAT AMPLITUDE, AND SIZE IN JACK MACKEREL, *Trachurus symmetricus*, AND OTHER FISHES

JOHN R. HUNTER AND JAMES R. ZWEIFEL[1]

ABSTRACT

The tail beat frequency and tail beat amplitude of jack mackerel, *Trachurus symmetricus*, 4.5 to 27.7 cm were measured at speeds of 15 to 212 cm/sec. Tail beat amplitude was a constant proportion of length at all speeds but tail beat frequency changed with speed; thus speed depended only on frequency of the tail beat and length. A simple mathematical model for estimating swimming speed from tail beat frequency and fish length was derived from the *Trachurus* data and applied to data for three marine fish — *Scomber japonicus*, *Triakis henlei*, and *Sardinops sagax* — and to data for freshwater fish from the literature. The general form of the model was $V - V_0 = L(KF - F_0)$ where V is fish speed, V_0 is length-dependent minimum swimming speed at minimum tail beat frequency F_0, and L is fish total length. The model represented a major improvement over previous equations because it provided an unbiased correction for length, was sensitive to specific differences, and provided a more accurate estimation of speed.

Of the variables that determine the swimming speed of a fish, the size of the fish, the frequency of the tail beat, and the amplitude of the tail beat are among the most important. Knowledge of the relationships between swimming speed and these variables is important not only for an understanding of the mechanism of locomotion in fish but because it may be used to forecast maximum swimming speeds (Bainbridge, 1958), to estimate swimming speeds indirectly by analysis of tail beat frequencies, and possibly to estimate fish size and make specific identifications of fish targets with doppler Continuous Transmission Frequency Modulated sonar (Hester, 1967).

Bainbridge (1958) described the relationship between tail beat frequency, tail beat amplitude, and size for three species of freshwater fish: dace, *Leuciscus leuciscus*; trout, *Salmo gairdneri* (*S. irideus*); and goldfish, *Carassius auratus*. He concluded that the amplitude of the tail beat increased with the tail beat frequency to about 5 tail beats/sec and thereafter became constant. Speeds above 5 beats/sec were dependent only on the frequency of the tail beat and the length of the fish. The relationship between speed, frequency, and length above 5 beats/sec was nearly the same in the three species studied; consequently, he used a single equation to express this relationship for all three species. No similar study exists for marine fish although some measurements of tail beat frequency and amplitude have been made incidental to other studies. Yuen (1966) measured the tail beat frequency of skipjack tuna, *Katsuwonus pelamis*, and yellowfin tuna, *Thunnus albacares*, from cine photographs taken from the viewing port of a research vessel and Magnuson and Prescott (1966) measured the tail beat frequency of Pacific bonito, *Sarda chiliensis*, from cine photographs taken through a window in an oceanarium. The slopes of the lines relating tail beat frequency to speed in body lengths per second for skipjack and yellowfin tunas and bonito were sufficiently different from those of Bainbridge (1958), for Hester (1967) to speculate that species might be identifiable by this relationship. The measurements were taken from lateral photographs of free-swimming schools; thus the tail beat amplitude and the absolute

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size of the fish were not measured and tail beat frequency was measured over a limited speed range. Fierstine and Walters (1968) measured both tail beat frequency and amplitude of wavyback skipjack, *Euthynnus affinis*, from dorsal cine photographs of free-swimming fish in circular swimming pools but only five, one-beat sequences of swimming were analyzed.

The objective of the present study was to determine the relationships between swimming speed, fish length, tail beat amplitude, and tail beat frequency in a pelagic marine fish, jack mackerel, *Trachurus symmetricus*. To accomplish this objective, dorsal cine photographs were taken of fish swimming in currents of different speeds in a specially designed activity chamber. For comparative purposes tests were also run on three other marine fish: chub mackerel, *Scomber japonicus;* Pacific sardine, *Sardinops sagax*; and a shark, *Triakis henlei*.

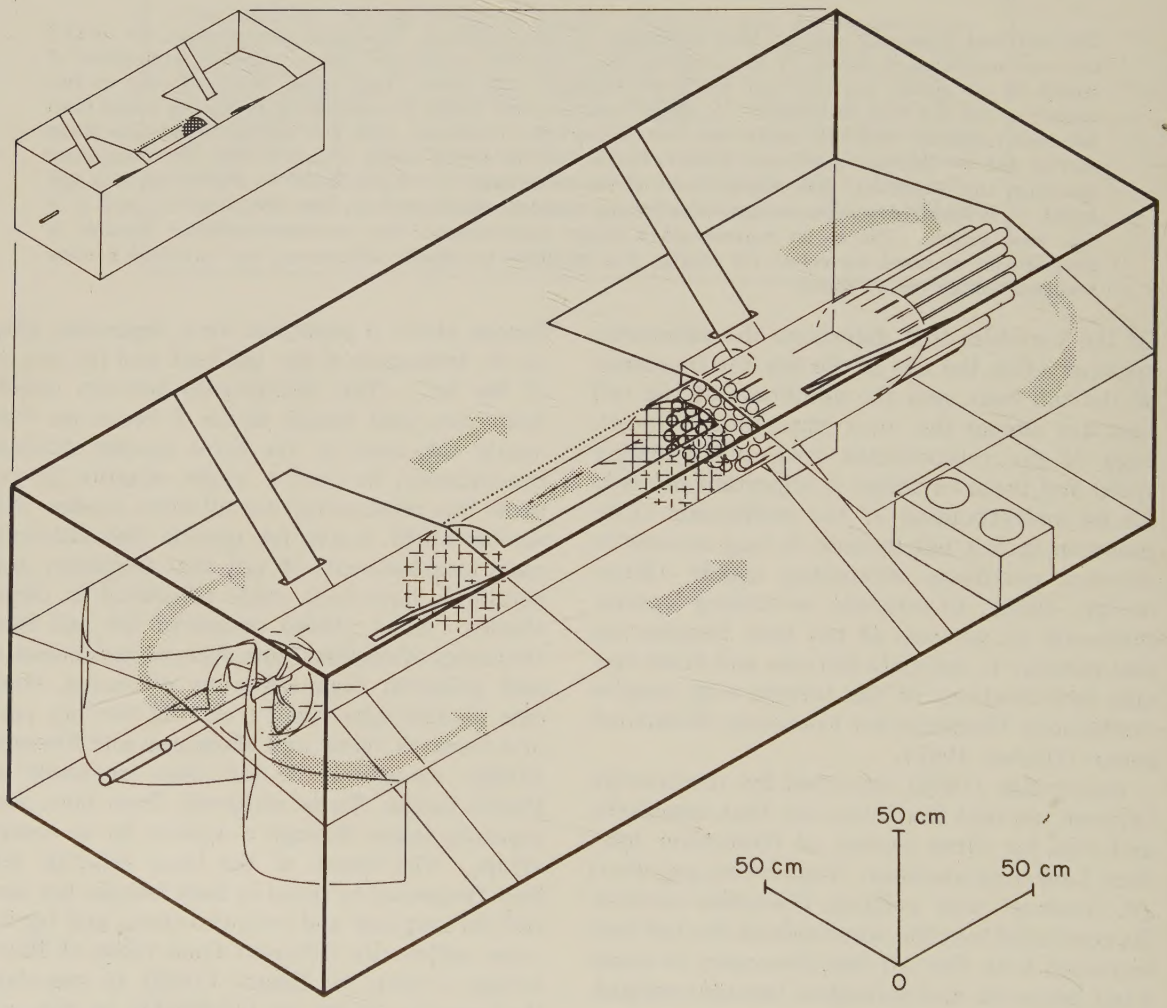

FIGURE 1.—Apparatus used to measure swimming speeds of fishes. Inset upper left apparatus shown with opaque walls; center, isometric, three dimensional scale drawing, walls, deflectors and other structures are shown as transparent for purposes of illustration; arrows indicate direction of current flow; and lower right, scale for vertical and horizontal planes of drawing.

APPARATUS

Swimming speeds were measured in an activity chamber (Figure 1) built after that of Beamish (1966). A fiber glass tube 230 cm long and 41 cm in diameter was immersed in an open bath. An 80-cm section of tube was the swimming compartment. The compartment had metal screens at the ends and a transparent acrylic plastic hatch which conformed to the contours of the tube. The walls of the tube within the swimming compartment were white and had black stripes spaced at 5.0-cm intervals to provide a visual reference for the swimming fish. Water velocity in the chamber was regulated by the speed of a 39-cm propeller driven by a variable speed 50-hp motor and by changing the screens at the two ends of the swimming compartment. Water was drawn into the compartment from the bath over deflectors, and through baffles and screens. The screens, baffles, and deflectors reduced turbulence and provided water of relatively uniform velocity throughout the swimming compartment. Their arrangement and design were determined empirically by measurement of the horizontal and vertical distribution of flow in the chamber.

The speed range of the apparatus was 12 to 212 cm/sec. The full range was obtained by changing the screens at the ends of the swimming compartment. A velocity range of 12 to 69 cm/sec was obtained when screens of 39% open area were used, one of 15 to 139 cm/sec for screens of 56% open area, and one of 19 to 212 cm/sec for screens of 75% open area.

A digital voltmeter measured to the nearest millivolt the voltage produced by a voltage generator attached to the propeller shaft. The voltage produced by the generator was proportional to propeller revolutions and was used to regulate them. An impeller flowmeter (Marine Advisers Inc., Model B-7C)[2] was used to relate propeller revolutions in volts to water flow in the apparatus. The meter sampled an area 7.6 cm in diameter and had an accuracy of ± 2.5 cm/sec when moved through static water at a known speed (for a description of the meter and a calibration curve see Olson, 1967).

Propeller revolution was related to water speed in the chamber by three series of calibrations, one for each of three screen types used. Nine to 19 different speed levels were measured in each series of calibrations. More levels were required for slow speed ranges than for fast ones because the response of the flowmeter was nonlinear at low speeds. At each level water speed was measured at 12 different radial positions midway in the swimming compartment. The speed of the water at a given level was the average of the 12 measurements, adjusted for the extent of the area sampled by the meter (Tranter and Smith, 1968). Variation among the 12 sampling points did not exceed ±10% of the mean speed and was usually much lower. The relationship between mean water speed in the chamber and propeller revolutions was linear, and the error in estimating the mean water speed from revolutions did not exceed ± 0.2 cm/sec. Thus, the principal sources of error in estimating the speed of the water in which a fish swam were the possible 10% variability in flow within the chamber and the ± 2.5 cm/sec accuracy of the flowmeter.

Fish swimming in the compartment were assumed to be swimming at the mean speed of the water in the compartment. They were photographed from above with a 16 mm high-speed motion picture camera at speeds of 64 to 200 fps. Camera speed was adjusted to provide about 10 frames per complete tail beat. A viewing box floated on the water surface above the swimming compartment to eliminate distortion in the photographs caused by ripples.

METHODS

Film was analyzed by use of a coordinate reader and digitizer (Hunter, 1966) and a computer program was used to calculate tail beat frequency and tail beat amplitude from the digitized information. One tail beat was one complete oscillation of the tail, and the tail beat frequency was the number of beats per second. Tail beat amplitude was the distance in centimeters between the lateral most excursion of

[2] Reference to commercial products does not imply endorsement.

the tip of the tail measured perpendicular to the axis of progression plus the mirror image of that measurement on the other side of the axis of progression.

The film sequences selected for analysis were usually ones in which the fish held a constant position in the current and swam steadily. Occasionally at higher speeds it was not possible to obtain such a sequence because the fish did not maintain a constant position but rather accelerated and decelerated. In this case a sequence was chosen in which no net movement existed between the beginning and end of the sequence although the fish moved slightly forward and backward within the sequence. Usually 5 complete tail beats were analyzed per speed level but occasionally as few as 2.5 and as many as 11 were analyzed.

Sixteen speed levels were used in the experiments; seven of the levels, 15 to 60 cm/sec, were graduated at intervals of 25% of the preceding level and nine of the levels, 69 to 212 cm/sec were graduated at intervals of 15%. A speed level interval greater than 10% was used because of the possible 10% variability in flow within the swimming compartment.

A grand total of 176 speed tests was analyzed for 14 jack mackerel, varying in total length from 4.5 to 27.7 cm. Owing to the differences in length, no fish was able to swim at all levels. All 14 fish swam at five levels, 24, 30, 38, 48, and 60 cm/sec, and all but the two smallest fish swam at the next five higher levels, 79, 91, 105, 121, and 139 cm/sec. Only fish of 16 cm or larger were tested at speeds above 139 cm/sec and only those less than 16 cm were tested at 15 cm/sec.

Other species were tested for comparative purposes but fewer observations were made. Seventy-four swimming sequences of five *Scomber*, 26.3 to 32.2 cm total length, were analyzed, nine sequences of five *Sardinops*, average length 13.6 cm, and seven sequences of one *Triakis*, 23.6 cm.

All fish were tested singly except for *Sardinops*, which was tested in a group of five. Fish were held in the swimming compartment at a low speed for about 30 min before an experiment began. Seawater temperature ranged from 17.0° to 19.5° C among experiments but did not vary over a degree within an experiment.

RESULTS

The tail beat amplitude of *Trachurus* did not change with speed but was constant at all speed levels and was directly related to length (Figures 2 and 3). Tail beat frequency, on the other hand, changed with speed and, therefore, was the only speed modulator measured in these experiments. In all species studied the relationship between tail beat frequency and velocity

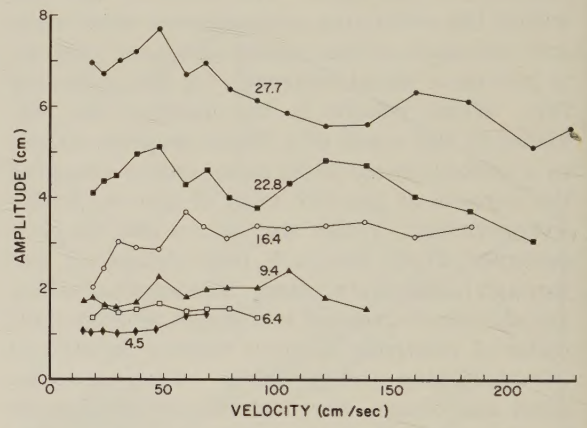

FIGURE 2.—Tail beat amplitude at various speeds for six *Trachurus*, 4.5 to 27.7 cm total length.

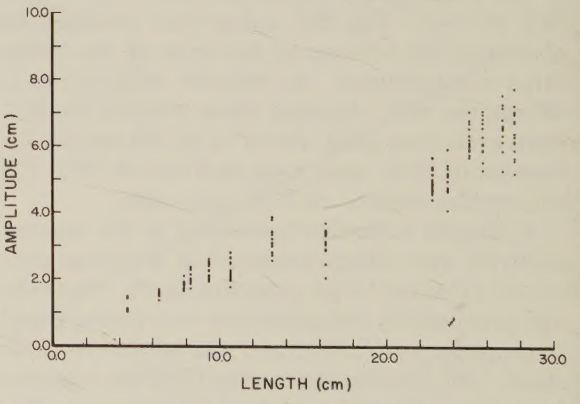

FIGURE 3.—Relationship between tail beat amplitude and total length for 14 *Trachurus*, 4.5 to 27.7 cm total length. ($A = 0.23177L$, $s_{yx} = 5.068$, and $N = 176$.)

was linear throughout the range of test speeds, but the slope and intercept of the regression lines varied with fish length (Figure 4, Table 1).

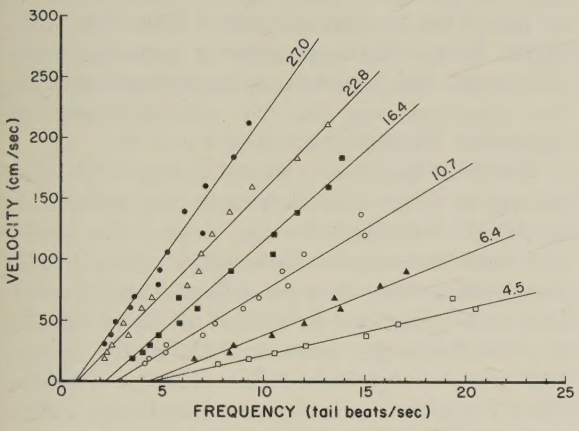

FIGURE 4.—Relationship between speed and tail beat frequency for six *Trachurus*, 4.5 to 27.0 cm total length. Equations for regression lines shown in figure are given in Table 1.

TABLE 1.—Standard deviation (s_{yx}), intercept (a), and slope (b) for regression of speed (cm/sec) on tail beat frequency and slope and intercept for regression of speed/length on frequency for *Trachurus*.

Length (cm)	N	Speed (cm/sec) on frequency			Speed/length on frequency	
		a	b	s_{yx}	a	b
4.5	8	−17.786	3.996	3.889	−3.952	0.888
6.4	9	−29.761	6.838	4.284	−4.650	1.068
7.9	11	−37.527	8.326	4.135	−4.624	1.054
8.3	11	−39.736	9.374	10.096	−4.787	1.129
9.4	13	−35.241	8.953	5.258	−3.749	0.952
10.7	13	−29.012	10.431	5.612	−2.781	0.975
13.2	13	−19.860	11.514	10.762	−1.504	0.873
16.4	14	−32.541	14.780	9.060	−1.984	0.901
22.8	15	−15.407	17.497	6.945	−0.676	0.767
23.7	13	−13.723	19.753	6.457	−0.579	0.833
25.0	15	−33.403	23.394	11.832	−1.336	0.936
25.8	13	−23.220	21.730	8.734	−0.900	0.842
27.0	13	−20.521	23.960	9.157	−0.760	0.887
27.7	15	−11.240	20.509	9.182	−0.406	0.740
Total	176					

The length-dependent differences in intercept were probably a function of differences in minimum speed and minimum tail beat frequency. Fish have a minimum tail beat frequency and a minimum swimming speed below which they cannot swim by movement of the caudal fin and these minima were a function of body length. In the past, speed was scaled directly to length; that is, speed was divided by length and regressed on frequency (Bainbridge, 1958; Magnuson and Prescott, 1966; Yuen, 1966). Our data suggest, however, that division of speed by length would introduce bias because of the existence of a minimum speed and tail beat frequency different from zero, the dependence of minimum speed on length, and possible length-dependent differences in the slope of the regression of speed on frequency. For example, when we divided speed by length, size-dependent differences in intercept and possibly the slope still existed (Table 1, last two columns). In addition a curvilinear trend is introduced at low speeds in the combined data because of the lack of an intercept (minimum speed) correction. Thus an equation that relates speed to tail beat frequency for fish of different length must include an adjustment for size-dependent differences in minimum swimming speed, minimum tail beat frequency, and perhaps also for size-dependent differences in the slope coefficient.

The existence of size-dependent variables introduces certain problems in the interpretation of these data because of the possibility that specific differences in size dependency may exist. For example, differences exist among species in the coefficients used to relate size to various swimming characteristics such as burst and sustained speeds (Bainbridge, 1960) but it is uncertain whether or not these differences reflect real specific differences or if they are simply differing estimates of a common coefficient. Owing to the great variability inherent in swimming speed studies and because of the sensitivity of the length coefficients to the size range of animals in the sample, these two alternatives are equally plausible. In addition, specific differences in the relationship between size and swimming functions may also depend on the particular function considered (Bainbridge, 1960). For example, the coefficient relating size to maximum sustained speed may be different from the one that relates size to beat frequency or burst speed. Thus, species may differ from one another in the way each swimming function is related to size. If such specific differences exist

then direct comparisons between species are impossible, but if they do not exist then a general model can be derived from our data which can be used to make specific comparisons. In the interpretation of our data on *Trachurus* we will consider the alternatives, Case I where all swimming functions are related to size on a species-specific basis, and Case II where swimming functions are proportional to the same power of length in different species.

To evaluate Case I where length coefficients are considered to be species-specific we regressed speed on frequency by the general relationship.

$$V = \alpha_1 L^{\alpha_2} + \alpha_3 L^{\alpha_4} * F$$

where V is swimming speed in centimeters per second, F is tail beat frequency in beats per second, L is total length in centimeters and $\alpha_1 L^{\alpha_2}$ is the intercept function and $\alpha_3 L^{\alpha_4}$ is the slope function for the tail beat frequency-swimming speed relationship. Estimates were obtained by use of Marquardt's Algorithm for fitting nonlinear models (Conway, Glass, and Wilcox, 1970). For *Trachurus* the 90% support-plane confidence intervals (Conway et al., 1970) for α_3 and α_4 were $0.72 < \alpha_3 < 1.82$ and $0.72 < \alpha_4 < 1.01$ where $\hat{\alpha}_3 = 1.28$ and $\hat{\alpha}_4 = 0.86$.

To evaluate Case II where length coefficients are the same for all fish we assumed the slope coefficient α_4 equaled one. When $\alpha_4 = 1$, $\hat{\alpha}_3 = 0.86$ with 90% confidence limits of $0.79 < \alpha_3 < 0.91$. On the basis of the *Trachurus* data alone there seems to be little or no difference between the use of unity for the slope coefficient or use of the estimated value of 0.86. The similarity in the two estimates is apparent when the actual fish lengths are substituted into the two equations and the two sets of slopes are compared (Table 2, columns 1 and 2).

We fitted the Case I model to four additional species to determine the extent they differed in the length coefficient for the slope in the speed-tail beat frequency relationship. Used in this comparison were data we collected on *Scomber*, and data presented in scatter plots of velocity and frequency for individual *Carassius*, *Salmo*, and *Leuciscus* by Bainbridge (1958). We used the *XY* digitizer to transcribe Bainbridge's data onto cards. We may have failed to interpret correctly some of the overlapped points in his graphs but the effect of these errors on the statistical parameters we estimated would be negligible. We did not use the data presented by Magnuson and Prescott (1966), Yuen (1966), or Fierstine and Walters (1968) because in these studies the absolute speeds and the lengths of the fish were unknown.

Our estimates of the slope coefficient for the speed-tail beat relationship, $\hat{\alpha}_4$, varied from 0.76 in *Salmo* to 1.22 in *Carassius*, and the 90%

TABLE 2.—Slopes for the speed-frequency relationship for individual *Trachurus* when the general relationship is slope $= 1.28\ L^{0.86}$ (Case I) and when slope $= 0.86L$ (Case II); estimated minimum speed (V_0) for each fish when $V_0 = 0.80L^{2/3}$; lowest observed test speed (V_{obs}); the minimum tail beat frequency (F_0) estimated by substitution of V_0 the Case II equation for each fish; and the lowest observed tail beat frequency (F_{obs}).

Length (cm)	$b = 1.28L^{0.86}$ Case I	$b = 0.86L$ Case II	$V_0 = 0.80L^{2/3}$	V_{obs}	$F_0 = 3.98L^{-1/3}$ Case II	F_{obs}
4.5	4.67	3.87	2.19	14.9	2.41	7.77
6.4	6.32	5.50	2.77	18.9	2.14	6.60
7.9	7.57	6.79	3.19	24.0	2.00	6.75
8.3	7.90	7.14	3.30	24.0	1.97	6.68
9.4	8.79	8.08	3.59	15.1	1.88	5.22
10.7	9.83	9.20	3.92	15.0	1.81	4.18
13.2	10.98	11.35	4.51	15.0	1.68	2.91
16.4	14.19	14.10	5.21	19.0	1.56	3.56
22.8	18.84	19.61	6.50	18.9	1.40	2.18
23.7	19.48	20.38	6.67	24.1	1.38	1.86
25.0	20.39	21.50	6.91	19.8	1.36	2.46
25.8	20.95	22.19	7.06	24.7	1.35	2.11
27.0	21.78	23.22	7.28	31.4	1.33	2.16
27.7	22.27	23.82	7.40	19.0	1.32	1.78

support-plane intervals for $\hat{\alpha}_4$ in all species included unity (Table 3). Clearly if a common slope-length coefficient exists among these species, it must be close to 1. We conclude the assumption of unity for the slope-length coefficient is an acceptable practice and that it appears to introduce no significant bias in the species studied.

We now turn to the problem of estimation of the length-dependent coefficient for the intercept of the speed-tail beat relationship, that is, α_2. We noted previously that the biological significance of the existence of an intercept different from zero in the speed-frequency relationship may be that fish have a minimum speed below which they cannot swim by movements of the caudal fin. If we assume that the intercept is a function of the minimum swimming speed of a fish we can make an independent estimate of the intercept coefficient using an equation derived by Magnuson (1970) for estimation of the minimum swimming speed (V_0) of *E. affinis*. A somewhat simplified form of his equation is:

$$V_0 = \left(1 - \frac{D_e}{D_f} \right)^{1/2} * \left[\frac{g * Ma}{(C_{lf} A_{ft}) * \frac{p}{2}} \right]^{1/2}$$

where D_e is 1.025 (the density of sea water), D_f is the density of the fish, g is 980 cm/sec (the acceleration of gravity), Ma is mass of fish in air, C_{lf} is the coefficient of lift for the pectorals (assumed to equal 1), A_{ft} is the total lifting area of the extended pectoral fins in square centimeters, and p is the density of sea water, 1.025 g/cc. If we let $Ma = 0.004407 L^{3.21215}$ (the length-weight relationship for *T. symmetricus*, $N = 264$, unpublished data, Na-

TABLE 3.—Estimates of length coefficients for slope and intercept for five species of fish.

Species	$V = \alpha_1 L^{\alpha_2} + \alpha_3 L^{\alpha_4} * F$	90% support-plane interval[1]
Trachuhus symmetricus	$V = -44.57L^{-0.24} + 1.28L^{0.86} * F$	$-111.18 < \alpha_1 < 22.65$ $-0.80 < \alpha_2 < 0.31$ $0.73 < \alpha_3 < 1.82$ $0.72 < \alpha_4 < 1.01$ $s_{vf} = 11.41$
Scomber japonicus	$V = -0.56L^{0.55} + 1.20L^{0.83} * F$	$-160.78 < \alpha_1 < 159.66$ $-84.14 < \alpha_2 < 85.25$ $-8.15 < \alpha_3 < 10.57$ $-1.46 < \alpha_4 < 3.13$ $s_{vf} = 21.42$
Leuciscus leuciscus[2]	$V = -3.36L^{0.40} + 0.80L^{0.98} * F$	$-14.95 < \alpha_1 < 8.24$ $-0.84 < \alpha_2 < 1.65$ $0.47 < \alpha_3 < 1.12$ $0.83 < \alpha_4 < 1.12$ $s_{vf} = 7.81$
Salmo gairdneri[2]	$V = -1.77L^{0.64} + 1.40L^{0.76} * F$	$-14.79 < \alpha_1 < 11.26$ $-0.70 < \alpha_2 < 2.97$ $0.33 < \alpha_3 < 2.46$ $0.52 < \alpha_4 < 1.00$ $s_{vf} = 14.16$
Carassius auratus[2]	$V = -0.12L^{1.52} + 0.40L^{1.22} * F$	$-0.89 < \alpha_1 < 0.64$ $-0.50 < \alpha_2 < 3.53$ $0.17 < \alpha_3 < 0.63$ $1.01 < \alpha_4 < 1.44$ $s_{vf} = 5.54$

[1] Simultaneous confidence intervals for all parameters (Conway, Glass, and Wilcox, 1970).
[2] Data from Bainbridge (1958).

tional Marine Fisheries Service, La Jolla, Calif.), Area $= 0.02811L^{1.9124}$ (lifting area of pectorals of *T. symmetricus* was equal to twice the pectoral fin area), and $D_f = 1.03$ (the density of *T. trachurus* from Alexander (1959b), a closely related species to *T. symmetricus*), we obtain an estimate of $V_0 = 0.86L^{0.65}$ for *Trachurus*. This value is considerably below $10L^{0.50}$ obtained for *Euthynnus* by Magnuson (1970). The minimum swimming speed of *Trachurus* would be expected to be lower than that of *Euthynnus* because *Euthynnus* lacks a swim bladder and has a high specific gravity. Indeed, the minimum speed of *Euthynnus* is close to the endurance speed of many fishes with swim bladders (Magnuson, 1970).

In all other species except *Carassius* estimation of minimum speed was not possible because we had few or no estimates of the variables required in Magnuson's equation. In *Carassius* we used the specific gravity for carp, 1.002 g/cc (Alexander, 1959a), the pectoral fin area relationship of Area $= 0.02811L^{1.9124}$ from data we collected on seven *Carassius* 4.6 to 22.5 cm total length (the lifting area of the pectorals equaled twice the pectoral fin area) and the length-weight relationship of $Ma = 0.0065L^{3.20}$ for the seven *Carassius*. The estimate of minimum swimming speed V_0 for *Carassius* from these data was $0.87L^{0.65}$. This estimate was nearly the same as the one estimated above for *Trachurus* and it had the same coefficient of length. Thus, in *Trachurus* and in *Carassius* the minimum speed coefficient of length or, in our equation, the intercept coefficient $\hat{a}_2$, was 0.65.

That the length coefficient for the intercept was the same in *Carassius* and *Trachurus* supports the basic assumption of the Case II model, that is, the existence of common length coefficient among different species. To further test this assumption we estimated the intercept coefficient by fitting the combined data from all five species listed in Table 3 to the reduced Case II model

$$V = \alpha_1 L^{\alpha_2} + \alpha_3 L * F$$

The estimate α_2 for the combined data was 0.68 with 90% confidence limits of $-0.56 < \alpha_2 < 1.92$. Although the limits were wide, the estimate was very close to the other independent estimate for *Carassius* and *Trachurus* and suggests that the true value may be close to 2/3.

The evidence we presented supported the use of the Case II equation and the use of 2/3 for the length-dependent coefficient for the intercept function and of unity for the length-dependent coefficient of the slope function. Thus, we fit the reduced Case II model

$$V = \alpha_1 L^{2/3} + \alpha_2 L * F$$

to the data from each of the five species listed in Table 3 and to that from two additional species *Triakis* and *Sardinops* for which we had a small number of observations. The resultant equations were useful nonbiased predictive models for the estimation of speed (V) from length (L) and tail beat frequency (F) in each species (Table 4). The regression lines for these equations do not pass through the origin, however. They cut the abscissa before zero and consequently the intercept terms are negative. We pointed out previously that we believe the existence of a negative intercept in the raw data implied that the fish had a minimum swimming speed below which they cannot swim by beating only the caudal fin. Thus, to make the model more biologically meaningful we adjusted the elevation of the intercept function to correspond to the theoretical minimum swimming speed, V_0. (It should be remembered that the length-dependent slope of V_0 was about the same as the length coefficient, α_2, and it was this similarity that led us to use 2/3 as the intercept coefficient.)

To express the Case II model in terms of minimum speed we used V_0 estimated from Magnuson's equation to solve for a minimum tail beat frequency, F_0, and expressed the final relationship in the form

$$\frac{V - V_0}{L} = KF - F_0.$$

In species other than *Trachurus* and *Carassius*, a theoretical estimate of V_0 was not possible and consequently we assumed V_0 was propor-

TABLE 4.—Swimming speed-tail beat frequency equations for seven species of fish.

Species	$V = \alpha_1 L^{2/3} + \alpha_2 L * F$	90% support-plane interval[1]
Triakis henlei	$V = -1.39L^{2/3} + 0.93L * F$	$-2.71 < \alpha_1 < -0.01$ $0.77 < \alpha_2 < 1.09$ $s_{vf} = 2.49$
Trachurus symmetricus	$V = -2.50L^{2/3} + 0.83L * F$	$-3.28 < \alpha_1 < -1.59$ $0.78 < \alpha_2 < 0.88$ $s_{vf} = 12.39$
Scomber japonicus[2]	$V = -2.20L^{2/3} + 0.82L * F$	$-3.18 < \alpha_1 < -1.13$ $0.76 < \alpha_2 < 0.88$ $s_{vf} = 12.31$
Leuciscus leuciscus	$V = -1.58L^{2/3} + 0.74L * F$	$-2.21 < \alpha_1 < -0.86$ $0.71 < \alpha_2 < 0.78$ $s_{vf} = 7.78$
Carassius auratus	$V = -0.66L^{2/3} + 0.66L * F$	$-1.47 < \alpha_1 < 0.17$ $0.61 < \alpha_2 < 0.74$ $s_{vf} = 6.09$
Salmo gairdneri	$V = -1.28L^{2/3} + 0.64L * F$	$-2.63 < \alpha_1 < 0.13$ $0.59 < \alpha_2 < 0.69$ $s_{vf} = 17.72$
Sardinops sagax	$V = 0.49L^{2/3} + 0.50L * F$	$-1.61 < \alpha_1 < 2.58$ $0.39 < \alpha_2 < 0.62$ $s_{vf} = 5.77$

[1] Simultaneous confidence intervals for all parameters (Conway, Glass, and Wilcox, 1970).
[2] One deviant fish omitted; if fish included $V = -0.53L^{2/3} + 0.66L * F$, and $s_{vf} = 21.31$.

tional to $L^{2/3}$. The elevation of the line relating V_0 to length for a species was estimated by assuming that the lowest observed speed fell on that line. For *Trachurus* and *Carassius* we recalculated the elevation for a slope of 2/3.

Our estimates of V_0 and F_0 were not definitive. For *Trachurus* no fish were tested at speeds close to the theoretical minimum. Our estimates based on the theoretical minimum speed were closest to the observed minimum speeds in fish 9.4 cm total length and larger (Table 2) because in these larger fish the test speeds were sufficiently low for fish to swim with pectoral fins fully extended, an event that occurs near the minimum swimming speed (Magnuson, 1970). For *Carassius* the agreement between the theoretical estimate of minimum speed and observed minimum speeds was better (Table 5). The explanation for this is that the techniques used by Bainbridge (1958) permitted estimates at much lower speeds than the one we used. These data clearly show that in neither *Trachurus* (Table 2) nor *Carassius* (Table 5) was either V_0 or F_0 seriously overestimated. We feel that our estimates for these two species were reliable.

The fit to the general equation was good in all species (Figure 5, Table 6). The intercept for the regression line did not differ from zero and the scatter at low velocities was less than it was when no intercept correction was used (see figures in Bainbridge (1958) for comparison). The regression coefficient, K, in our equation differed among species. For the five species for which significant data were available, it was the highest in *Trachurus* and lowest in *Salmo*. Since amplitude was a constant, these results implied that the speed output per beat of the caudal fin was greater in *Trachurus* and *Scomber* than it was in *Salmo* and *Carassius*. In *Scomber*, the coefficient, K, may be uncertain because the values of one of the five fish tested deviated considerably from the rest. In Figure 5, all of the values to the right of the regression line above

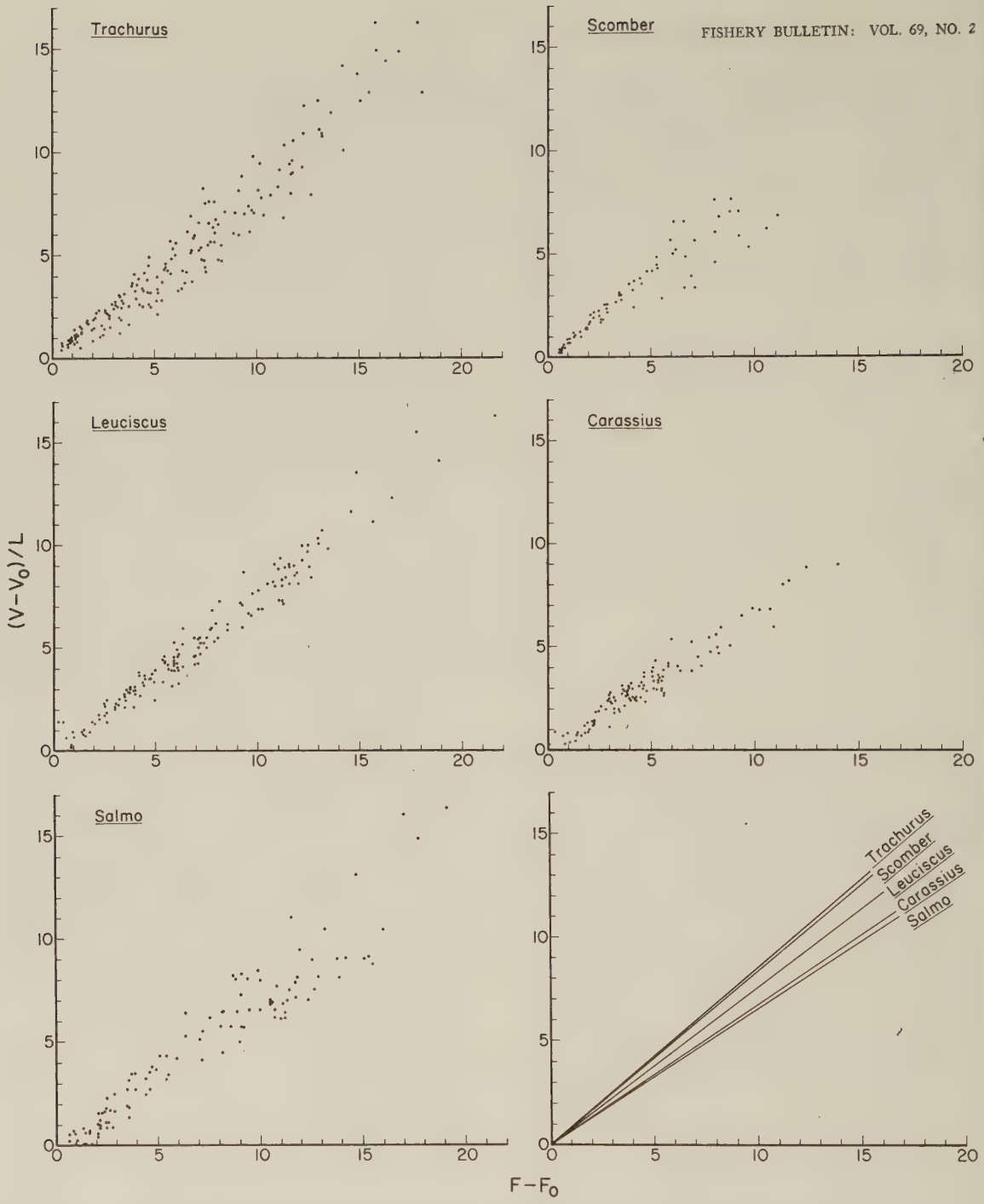

FIGURE 5.—Relationship between swimming speed corrected for minimum speed over length and tail beat frequency corrected for minimum frequency for *Trachurus* and *Scomber* from the present data, and for *Leuciscus*, *Salmo*, and *Carassius* from Bainbridge (1958). Graph at lower right shows individual regression lines for all above species, equations for lines are given in Table 6.

Table 5.—Slopes for the velocity-frequency relationship for individual *Carassius* studied by Bainbridge (1958) when the general relationship is slope $= 0.68L$; estimated minimum speed when $V_0 = 0.81L^{2/3}$; observed minimum swimming speed (V_{obs}); the tail beat frequency F_0 estimated by substitution of V_0 into the corrected slope equation; and the lowest observed tail beat frequency (F_{obs}).

Length (cm)	$b = 0.66L$	$V_0 = 0.81L^{2/3}$	[1]V_{obs}	$F_0 = 2.22L^{-1/3}$	F_{obs}
4.6	3.04	2.25	3.50	1.34	1.30
7.0	4.62	2.98	9.30	1.17	1.52
9.5	6.27	3.66	11.60	1.04	2.03
15.2	10.03	5.01	33.70	0.90	3.38
22.5	14.85	6.52	13.70	0.78	1.63

[1] Data from Bainbridge (1958).

TABLE 6.—Minimum speed (V_0), minimum tail beat frequency (F_0), the coefficient K in equation $V - V_0 = L(KF - F_0)$ arranged in order of K.

Species	N	V_0	F_0	K
Triakis henlei	6	$0.15L^{2/3}$	$1.66L^{-1/3}$	0.93
Trachurus symmetricus	176	[1]$0.80L^{2/3}$	$3.98L^{-1/3}$	0.83
Scomber japonicus	[2]61	$1.31L^{2/3}$	$3.51L^{-1/3}$	0.82
Leuciscus leuciscus[3]	149	$0.67L^{2/3}$	$3.04L^{-1/3}$	0.74
Carassius auratus[3]	111	[1]$0.81L^{2/3}$	$2.22L^{-1/3}$	0.66
Salmo gairdneri[3]	109	$0.52L^{2/3}$	$2.81L^{-1/3}$	0.64
Sardinops sagax	9	$2.23L^{2/3}$	$3.48L^{-1/3}$	0.50

[1] V_0 theoretical estimate based on equation of Magnuson (1970).
[2] One deviant fish omitted; if fish included, $N = 74$, $K = 0.66$.
[3] Original data from Bainbridge (1958).

4 beats/sec on the abscissa were from this single deviant fish. If the deviant fish is included $K = 0.66$, but if not, $K = 0.82$. We are inclined to use $K = 0.82$ because the values for the four fish were very similar and the protocol indicated that the deviant fish may have been overly fatigued when tested. *Triakis* appears to have a relatively high coefficient but not too much significance can be attached to the exact value for *Triakis* or for *Sardinops* because these were based on so few measurements.

In sum, the speed-tail beat equation (Case II) —Table 6—was biologically as well as statistically relevant, was sensitive to specific differences in swimming behavior, provided an unbiased correction for length, and made possible a more accurate estimation of swimming speed from tail beat frequency than heretofore has been possible.

TAIL BEAT AMPLITUDE

We pointed out previously that tail beat amplitude was a constant and was directly proportional to length and consequently the size coefficients for amplitude are probably the same as those for length. Thus amplitude (A) in centimeters can be substituted for length in the original Case II equation $V = \alpha_1 A^{2/3} + \alpha_3 L * F$. When this was done for *Trachurus* using all individual amplitude values ($N = 176$), we obtained the equation; $V = -6.5767A^{2/3} + 3.5637A * F$. The amplitude coefficient may be also estimated by substitution of the amplitude-length relationship for *Trachurus* ($A = 0.23177L$), into the Case II equation.

The tail beat amplitude data collected by Bainbridge (1958) were insufficient for specific estimates of an amplitude coefficient. The mean amplitudes for each of the fish we studied and for each of those studied by Bainbridge were nearly the same, when adjusted for body length. Variation within a species was as great as that between species (Figure 6). The relationship

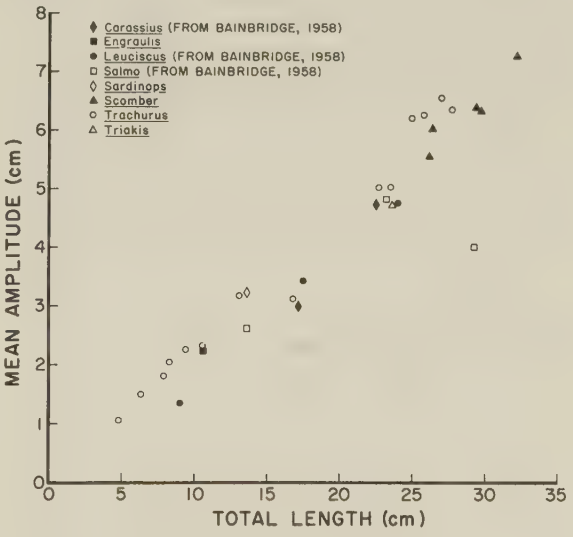

FIGURE 6.—Relationship between mean tail beat amplitude and length for every fish we studied and all those studied by Bainbridge (1958), $A = 0.21L$.

between mean tail beat amplitude and length for all species combined was $A = 0.21L$.

DISCUSSION

In all previous studies speed was divided by length then related to tail beat frequency. In our data when speed was converted to body lengths per second the relationship between speed and frequency was nearly identical to that given by Bainbridge (1958) for *Carassius*, *Leuciscus*, and *Salmo*. The confidence intervals for the slopes in the speed-frequency relationship in *Euthynnus* and *Thunnus* (Yuen, 1966) and in *Sarda* (Magnuson and Prescott, 1966) overlap the slope in the Bainbridge equation and the ones for *Trachurus* and *Scomber* when the body length conversion is used. Thus, when speed is in body lengths per second, the relationship between it and frequency is about the same in all fish studied to date from goldfish to mackerel and is adequately described by the Bainbridge equation $V/L = bf$. Thus the Bainbridge equation provides a description of the average relationship for fish in general but little significance can be attached to specific differences in slope. If more than a rough estimate of speed is required or if specific differences are important, or if estimates are needed near the minimum swimming speed it would be necessary to use the equation developed in this study.

Bainbridge (1958) concluded from his data that the frequency-speed relationship was curvilinear below a frequency of about 5 beats/sec because fish modulated their tail beat amplitude. His evidence for this conclusion was that in some fish amplitude appeared to decrease at lower frequencies, and that the distance per beat, calculated by dividing speed by frequency, declined at frequencies below 5 beats/sec but was constant above that frequency. His evidence for amplitude modulation at low speeds was weak. In the three *Salmo* studied no trend existed; in *Carassius* he suggested there might be a decrease in amplitude in one of the two fish studied, and in one of the two *Leuciscus* studied a trend existed slightly stronger than the one in *Carassius*. In sum, the evidence for a decline in amplitude measurements was based on possible trends in two of the seven fish studied. Two fish could easily give a misleading picture of the general trend in the data, especially when the variability in amplitude measurements are considered. In our studies we measured the tail beat amplitude in every fish at all possible speed levels and no evidence existed for a consistent change in amplitude with speed.

In Bainbridge's data the departure of distance traveled per beat from a constant at low frequencies was caused by the division of speed by frequency. Had the line relating frequency to speed passed through the origin, no bias would have existed but because the line intersected the abscissa at about 1 beat/sec division by frequency produced an artificial curvilinear trend at lower frequencies. We produced the same trend in distance per beat in our data by dividing speed by frequency but the trend was eliminated when a correction for the intercept was used. Thus the curvilinear trend in distance per beat in Bainbridge's data was an artifact caused by the method of calculation and consequently distance per beat was a constant at all frequencies. In addition, the apparent nonlinearity below 5 beats/sec in his graphs relating speed divided by length to frequency was also the result of the same intercept problem. Therefore, no evidence exists for consistent amplitude modulation at any speed range and speed appears to be related only to tail beat frequency and length in the species studied by Bainbridge (1958) as well as in the ones we studied. We concluded that during steady swimming at any speed, tail beat amplitude is a constant proportion of body length of the order of 0.21 L.

That the mean amplitude during steady swimming was constant does not mean that amplitude is not modulated under certain conditions. It is widely known that fish modulate tail beat amplitude when they accelerate (Gray, 1968). Further, we had the impression that some of the variability in the speed-frequency relationship was caused by differences in amplitude. These differences were infrequent and irregular in occurrence and consequently we were not able to evaluate them statistically. We are inclined to believe, however, that fish occasionally made minor adjustments in amplitude and frequency over the entire range of speeds, but these adjust-

ments were merely individual deviations from the general relationship we have described.

We do not wish to detract from the original and important contribution of Bainbridge (1958), by emphasis on the differences between his and our conclusions. His basic conclusions and equations were not greatly different from our own. We were able to examine more closely the form of the relationships he described because of a larger sample size made possible by the availability of automatic film analysis equipment and because of the existence of his data in the literature.

The question of species-specific size effects remains unresolved. In our general model a good fit was obtained in seven species when the minimum stalling speed was proportional to $L^{2/3}$, the frequency at this minimum speed was proportional to $L^{-1/3}$, and the slope coefficient was proportional to $L^{1.0}$. A comparative study on speed-related size effects in fishes would certainly be of value.

It also remains to be resolved whether or not it was appropriate to apply the minimum swimming speed equation developed by Magnuson (1970) for *Euthynnus affinis*, a fish that lacks a swim bladder, to such a broad assortment of species. The equation implies a functional relationship between minimum speed and hydrostatic equilibrium and implies existence of negative buoyancy at minimum speeds. We do not know if these relationships exist in all species; nevertheless his equation did provide a reasonable estimate for minimum speed and it functioned well in our equation.

The relationship between swimming speed and tail beat frequency we have described could be used in any application where it is necessary to measure swimming speeds of fish. For example, a sonic internal tag could be developed that telemetered tail beat frequency and thus the speed of free-swimming fish could be monitored continuously over extended periods.

The tail beat frequency-speed relationship could be used for size or species identification using Continuous Transmission Frequency Modulated sonar as suggested by Hester (1967). The increase of speed with frequency (our K value) varied from species to species and thus might be used for identification. If size were known, the minimum observed velocity would provide additional information for identification. Alternatively, if the species were known, minimum (or maximum) speed would provide an indication of size. The equation could also be used to estimate size from tail beat amplitude, but caution should be exercised because in our study amplitude was not modulated and consequently, we do not know whether or not speed and tail beat amplitude are linearly related within an individual.

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SUSTAINED SPEED OF JACK MACKEREL, *Trachurus symmetricus*

JOHN R. HUNTER[1]

ABSTRACT

Jack mackerel, *Trachurus symmetricus*, were forced to swim for up to 6 hr at various speeds in an activity chamber. The probit estimate for the swimming speed at which 50% of *Trachurus* would fatigue during 6 hr was 93.4 cm/sec (8.4 *L*/sec) for fish 10.0 to 11.9 cm and was 22.4 $L^{0.6}$/sec for fish 9.0 to 17.6 cm where *L* is the total length of the fish in centimeters. At higher speeds, *Trachurus*, 15 cm, swam for 3 min at 160 cm/sec or 10 *L*/sec. The swimming speed at which 50% fatigued declined exponentially with time for about the first 22 min of swimming and thereafter declined linearly with time. The possible significance of the time-speed relationship for *Trachurus* is discussed.

Although a substantial literature on the swimming speed of fishes exists (see Bainbridge, 1958; Gray, 1968), few reliable estimates of maximum sustained speed exist. Much of the literature on swimming speed of fishes is concerned with estimates of maximum speed or burst speed, that is, speeds that can be maintained for only a few minutes or less. A sustained speed implies, on the other hand, that the animal is capable of swimming at that speed for hours. For example, Brett (1967) recommended a minimum of 200 min for a fixed sustained speed test. Fairly wide agreement exists that 2 to 3 *L*/sec can be maintained for an hour or more and salmonids and herring seem capable of sustaining 3 to 4 *L*/sec for such periods (Blaxter, 1969). These conclusions were drawn primarily from studies of freshwater fish and salmon; no estimates of maximum sustained speeds have been made for fast-swimming pelagic marine forms. The object of this study was to determine the sustained speed threshhold of jack mackerel, *Trachurus symmetricus*, a pelagic marine fish of commercial importance. The body form and musculature of *Trachurus* appear to be designed for greater hydrodynamic efficiency at high speeds than other species heretofore studied. In *Trachurus*, lateral musculature is concentrated in the anterior portion of the trunk, and inserts by tendons on a small deeply forked caudal fin.

In addition to the interest in comparing the sustained speed capabilities of *Trachurus* with that of fish with other body forms, sustained speed data have significance in prediction of migratory capabilities and physiological limits.

APPARATUS AND METHODS

The apparatus used in the experiments was an activity chamber provided with a water current of various calibrated speeds. The apparatus was the same as the one described and figured by Hunter and Zweifel (1971) in this issue except that a port was provided in the transparent hatch of the swimming chamber so that fatigued fish could be removed by hand from the downstream screen without reducing the flow in the chamber. The error in estimating the water speed in the swimming chamber did not exceed 10% and it was assumed that the fish were swimming at the estimated speed.

The experimental design was essentially the same as that used by Brett (1967) for determining the sustained speed threshhold for sockeye salmon, *Oncorhynchus nerka*. Fifty-five groups of five *Trachurus* (9.0 to 17.6 cm total length, mean = 12.43 ± 0.11 cm) were subjected to a fixed speed of 38 to 160 cm/sec for 360 min or longer after an introductory period of about 30 min at a low speed. A time-lapse camera photographed the fish at 1-min intervals and the time to fatigue for each fish was determined from the photographs. The temperature of the water in the activity chamber and in the holding

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tank (a plastic swimming pool 15 ft in diameter) was maintained by a temperature regulation system at about 18.5° C. The mean test temperature was 18.48 ± 0.03° C. The fish were captured near Santa Catalina Island, Calif., on 12 September 1969. Tests began 2 weeks later and ended on 21 November 1969. Fish were fed an abundant ration of chopped squid, anchovies, and frozen brine shrimp. Probit analysis, a statistical technique first applied to sustained speed data by Brett (1967), was used to estimate sustained speed threshholds.

Variability in length posed a problem in the analysis. Although all fish were from the same school, differences in length existed; also the fish grew in the course of the study. These differences were insufficient, however, to determine the form of the relationship between length and sustained speed. In general, the relationship between length and sustained speed for other species (Bainbridge, 1962; Brett, 1965), theoretical considerations (Gray, 1968; Fry and Cox, 1970), and relationships between length and other swimming capabilities (Magnuson, 1970), indicate that speed is proportional to a fractional power of length equal to about $L^{0.6 \pm 0.1}$. In addition, the minimum swimming speed of *Trachurus* was proportional to $L^{0.6}$ when estimated from Magnuson's equation (Hunter and Zweifel, 1971). In light of the above evidence it seemed preferable to use 0.6 as the coefficient of length, although unity has been commonly employed in cases where length coefficients were unknown. As an alternative to this procedure I also estimated the percent fatigued at different speeds in centimeters per second and in body lengths per second for a narrow length range (10.0 to 11.9 cm total length) where the effect of differences in length would be negligible.

RESULTS

Within a few minutes after *Trachurus* were placed in the swimming compartment they became quiescent, swam steadily, and remained in about the same position in the compartment throughout the test or until they became fatigued and fell against the rear screen. This was in contrast to some other species which did not swim steadily, but swerved and oscillated from side to side.

The relationship between water speed and percent fatigue had the normal sigmoid form of a dosage response curve (Finney, 1952). Probit estimate of the applied water speed at which 50% fatigue occurred in 360 min of swimming and the 95% confidence limits were 94.40 ± 5.15 cm/sec for *Trachurus* 10.0 to 11.9 cm total length, $N = 127$ (Figure 1, Table 1). Thus,

TABLE 1.—Swimming endurance of *Trachurus symmetricus* in cm/sec and in $L^{0.6}$/sec.

Length 10.0-11.9 cm					Length 9.0-17.6 cm		
Speed cm/sec	N	Percent fatigued	Length		Speed[1] $L^{0.6}$/sec	N	Percent fatigued
			Mean	SD			
71	8	0	10.69	0.61	15.9	3	0
78	16	13	10.71	0.47	16.9	10	0
85	16	31	10.84	0.67	17.8	14	0
92	14	50	10.97	0.54	18.7	17	6
99	21	62	11.23	0.43	19.7	18	17
106	21	76	10.85	0.56	20.6	23	39
113	15	100	11.41	0.42	21.5	39	31
120	8	100	11.27	0.45	22.5	42	67
138	8	100	11.20	0.51	23.4	19	58
Total	127				24.3	20	60
					25.3	29	72
					26.2	19	100
					27.2	9	100
					28.1	4	100
					29.0	3	100
					30.0	1	100
					30.9	3	100
					31.8	7	100
					32.8	10	100
					33.7	4	100
					Total	294	

[1] Total speed range divided into 20 equal intervals; speeds listed are midpoints of those intervals.

50% of *Trachurus* in this length range could be expected to sustain a speed of about 8.4 L/sec or 22.1 $L^{0.6}$/sec for 360 min. For all *Trachurus* ($N = 294$) the water speed at which 50% fatigue occurred after 360 min of continuous swimming and the 95% confidence intervals were 22.4 ± 1.2 $L^{0.6}$/sec. The first estimate, based on a narrow length range, and the second one, based on all data, were reasonably close. On the other hand, when all data were in the form $V/L^{1.0}$ the 50% threshold was 9.34 L/sec which is higher than the preceding estimates. Inspection of these data, however, showed that the coefficient for length clearly was less than one and that use of unity biased the estimate.

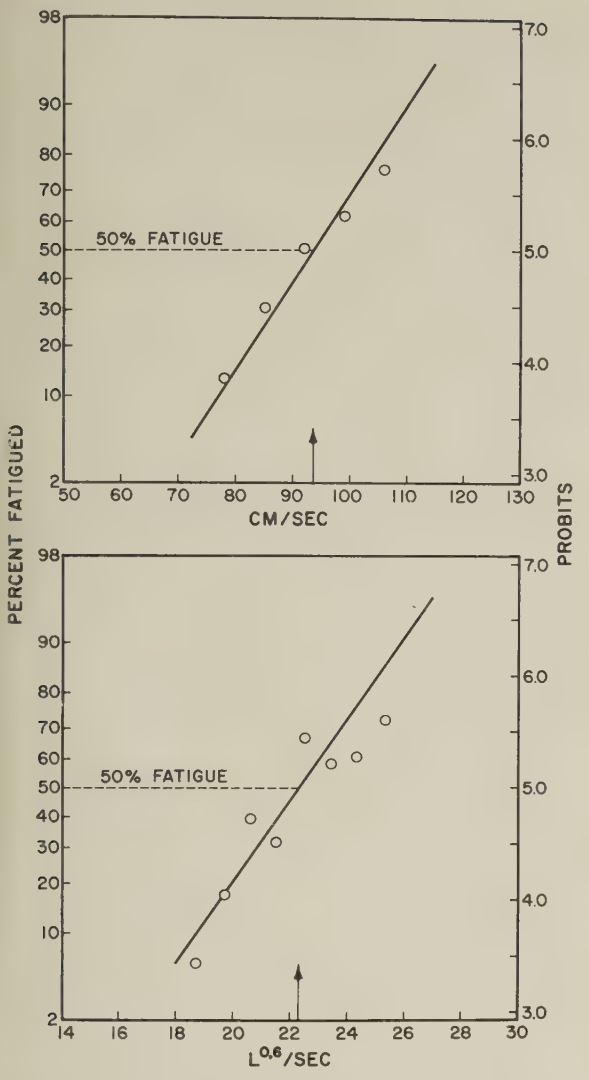

FIGURE 1.—Probit lines for sustained speed threshold for 6 hr of forced swimming at 18.5° C in juvenile *Trachurus symmetricus*. Upper panel, range fish length 10.0 to 11.9 cm $N = 127$, probit $= 0.077X - 2.238$; lower panel, range fish length 9.0 to 17.6 cm, $N = 294$, speed expressed in $L^{0.6}$/sec where L is the total length of the fish, probit $= 0.355X - 2.958$.

To determine the form of the relationship between the duration of the swimming period and the ability to maintain a certain speed, probit estimates of speed for five levels of fatigue were made for swimming periods varying from 10 to 360 min. The form of the relationship was about the same for all fatigue levels; speed estimates declined exponentially with time for short swimming periods and linearly with time for longer ones (Figure 2). The point

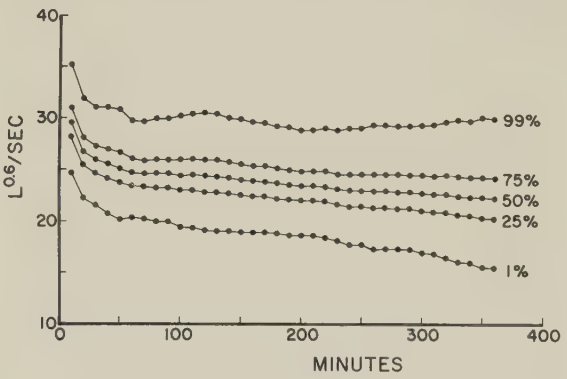

FIGURE 2.—Relation between speed in $L^{0.6}$/sec and the time it can be sustained for 1, 25, 50, 75, and 99 percent fatigue levels in *Trachurus symmetricus*. Estimates of speed for each fatigue level made at 10-min intervals of cumulated time.

of inflection from the exponential to the linear relationship was examined in detail for the 50% fatigue level. Probit estimates of the speed at which 50% of the fish fatigued were made for 2-min intervals of swimming cumulated over the first 100 min of observation. The data were plotted on semilog paper and a line fit by eye to the exponential function. The point of inflection appears to occur at about 22 min (Figure 3). Thus, speed at which 50% fatigued and the duration of the swimming period were exponentially related for durations up to about 22 min and were linearly related for longer periods of swimming.

The performance of juvenile *Trachurus* at high speed was of interest. Fifteen fish 14.6 cm mean total length (range = 13.4 to 16.6 cm) swam at the highest speed used in the study (160 cm/sec) for 2 to 6 min, mean time 3.4 min. Thus, *Trachurus* 15 cm total length were able to swim for about 3 min at about 10 L/sec or about 32 $L^{0.6}$/sec. A slightly higher level of

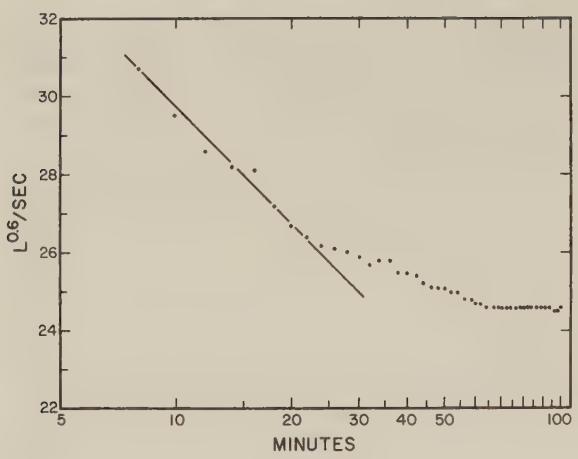

FIGURE 3.—Relation between speed at which 50% *Trachurus* fatigued and the duration of the swimming period. Duration of swimming period in minutes plotted on log scale to show exponential trend; line fit by eye. Speed estimates made at 2-min intervals of cumulated time over the first 100 min of swimming.

performance in length per second is obtained if we consider smaller fish. For example, fish of mean length 11.2 cm (length range 10.4 to 11.9 cm, $N = 8$) swam for 3 to 5 min (mean = 4.5 min) at 139 cm/sec or about 12 L/sec. This difference between large and small fish becomes negligible if 0.6 is used as a coefficient of length instead of unity because, as was pointed out previously, the length coefficient for *Trachurus* appears to be less than 1.

DISCUSSION

The exponential decline in swimming speed with time in fish is well documented; see for example Bainbridge (1960), Brett (1967), and Blaxter (1969). The general form of the relation between time and swimming speed in other fish resembles that for *Trachurus* although the speeds and endurance times are different in *Trachurus*. The physiological mechanisms responsible for the exponential relationship between swimming speed and endurance are generally believed to be the limited enery stores in the muscle, the rate these stores can be replaced and the rate catabolites are removed from the muscle (Bainbridge, 1960). A study by Pritchard, Hunter, and Lasker (1971) in this issue has provided an explanation for the form of the speed-time relationship in *Trachurus*. Pritchard et al. found that at speeds where an exponential relationship exists between time and speed the principal cause of failure of *Trachurus* was most likely the depletion of glycogen in the white muscle. On the other hand, fish that failed at speeds near the 6-hr 50% threshold, where a linear relationship exists between speed and time, had depleted not only the glycogen in the white muscle but that in the red muscle and liver as well. Thus, in *Trachurus* the form of the time-speed relationship could be explained on the basis of the extent of glycogen reserves available for locomotion and the time required to mobilize them from sites other than the white muscle. An exponential relationship between speed and time could be produced when the speeds are so high that the glycogen supply would be limited almost entirely to the white muscle because the supply in the white muscle would be used up and the fish would fail before significant amounts of glycogen could be mobilized from other sources. A linear relationship could exist where swimming speeds are sufficiently low that reserves in the white muscle could not be depleted before other sources in the red muscle and the liver are mobilized. We have, on one hand, a high rate of consumption using a more limited supply of fuel which could lead to an exponential relationship between speed and time and, on the other hand, a much lower rate of consumption using a relatively much larger fuel supply which could produce a linear relationship with time. An exponential relationship between energy consumption and swimming speed would enhance these effects.

Let us now consider the significance of the 6-hr sustained speed threshold determined for *Trachurus*. When compared with other determinations, this threshold appears to be unique because of different physiological mechanisms and because it is higher than those estimated for other fish. *Trachurus* at threshold speed appeared to use glycogen as fuel, white muscle for locomotion and maintained a high lactic acid

level in the muscle (Pritchard et al., 1971). These results are inconsistent with the conclusion that at sustained cruising speeds, fish use lipid metabolism to drive red muscle (Bone, 1966; Gordon, 1968; Blaxter, 1969) and that no oxygen debt is incurred (Brett, 1963). Reliance on glycogen as the principal fuel probably severely limits the time a speed can be maintained as compared with one where lipid metabolism is used exclusively. Thus the biochemical evidence indicates that the 6-hr speed threshold for *Trachurus* probably could be maintained only for a period of hours or perhaps days but certainly not weeks as one would expect if fat were used as fuel. The 6-hr threshold was also considerably above sustained speed thresholds for other fish where presumably fat may be employed as fuel. Brett (1967), in a study directly comparable with the current one, found the 50% fatigue time for sockeye salmon was 4 L/sec (about 11.3 $L^{0.6}$) whereas for comparable size jack mackerel it would be about 7.6 L/sec or 22.0 $L^{0.6}$. Other less comparable data give sustained or cruising speeds in the range of 3 to 4 L/sec (Blaxter, 1969). Thus, *Trachurus* has special physiological and structural adaptations that permit swimming for periods of hours at elevated speeds and it was the threshold for this swimming behavior that was measured. Other fishes, especially the scombroid fishes, may have similar abilities. For example, skipjack tuna can swim at 8 knots, or about 43 $L^{0.6}$, for over an hour (Commercial Fisheries Review, 1969) and yellowfin tuna and skipjack tuna have higher levels of white muscle glycogen than many other species of fish (Barrett and Connor, 1964).

It seems possible another speed threshold may exist for *Trachurus* below the present one where fat is the principal fuel, only red muscle is used for locomotion, and swimming can be maintained almost indefinitely. It would not be surprising if this lower threshold were closer to those determined for other fishes.

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THE TRANSPLANTING AND SURVIVAL OF TURTLE GRASS, *Thalassia testudinum*, IN BOCA CIEGA BAY, FLORIDA[1]

JOHN A. KELLY, JR., CHARLES M. FUSS, JR., AND JOHN R. HALL[2]

ABSTRACT

Turtle grass was transplanted to an unvegetated, dredged canal and a hand-cleared portion of a flourishing grass bed. Complete or partial success was attained in 7 of 14 methods used. The best method, in which short-shoots (rhizomes removed) were dipped in a solution of plant hormone (Naphthalene Acetic Acid) and attached to construction rods for transplanting, was 100% successful and may be suitable for general application.

Turtle grass, *Thalassia testudinum*, and other marine grasses are an invaluable asset to the marine ecosystem. They are primary producers and form an essential ecological niche in which a great number and variety of species find food and shelter. They are also important agents in the control of substrate erosion and the depositions of sediments (Stephens, 1966).

Uncontrolled dredging and filling of submerged lands have destroyed many turtle grass beds and their dependent fauna, some of which are economically important. An immediate need exists not only for sharply restricting further destruction of sea grass beds but also for replacing lost beds. One method of replacing them may be by transplanting sea grasses to areas that are suitable for their growth or to areas that are made favorable by soundly planned engineering (Phillips, 1960; Strawn, 1961). Areas surrounding spoil banks and finger-fill canals (dredged canals between filled land masses) would be suitable if they were constructed to supply zones of optimum depth for growth of marine grasses.

Unsuccessful earlier attempts to transplant turtle grass in Tampa Bay showed that the main problem was erosion by tidal currents. Turtle grass is buoyant, and new transplants tend to work free of the sediments and float to the surface when disturbed by water movement (Phillips, personal communication).[3] Another marine plant, eelgrass (*Zostera marina*), was transplanted successfully on the coast of Washington by Phillips (1967) and in the Aleutian Islands by Jones[4] and McRoy[5] (personal communication), but details on methods are not yet published. Successful growth of turtle grass under artificial conditions (Fuss and Kelly, 1969) led us to attempt transplanting it from one field location to another as described in the present paper.

Turtle grass spreads vegetatively by creeping rhizomes (long-shoots) buried in the substrate (Figure 1). Work by Tomlinson and Vargo (1966) showed that this growth is dependent entirely upon the vigorous activity of meristematic tissue in the apexes of rhizomes. The apex is also the only source of short-shoots (erect lateral branches) that develop from buds at this site. In the Miami area (Phillips, 1960) and tropical parts of its range, the plants also reproduce by flowering. Tampa Bay, however, is near the northern limit of the flowering capability of *Thalassia* (Phillips, 1960); thus, we

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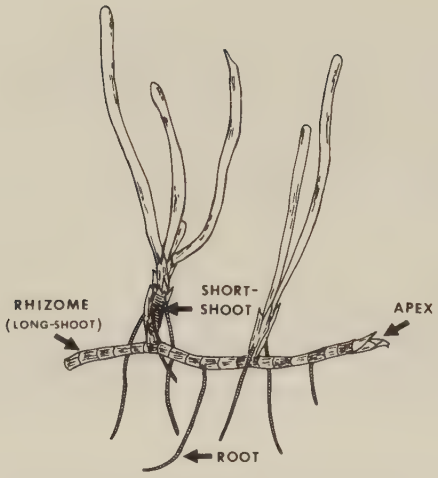

FIGURE 1.—External features of *Thalassia testudinum*.

confined our restoration studies to the transplantation of adult plants. This paper describes the procedures for and results of transplantation of turtle grass into modified environments.

DESCRIPTION OF TRANSPLANT SITES

All experiments took place in the southern end of Boca Ciega Bay, Fla., an elongate coastal lagoon joined to Tampa Bay and separated from the Gulf of Mexico by a line of barrier islands (Figure 2). The area it encompasses is a paramount example of grass bed destruction by hydraulic engineering (Hutton et al., 1956; Phillips, 1960; Taylor and Saloman, 1968).

A rectangular area 8.2 by 21 m (27 by 7 ft) in a large turtle grass bed was cleared by hand to serve as the control site. Two other transplant areas of the same size were in two adjacent finger-fill canals in a large land-fill development.

Construction of houses had not begun along the canals selected, and none was built during the experiments. Boating in the canals was light, and during periodic inspections we saw no disturbance of the plants directly attributable to man.

Sediments from transplant sites were analyzed by particle size. A sample from the control site was 95.5% sand (>62.5μ) and 4.5% silt and clay (<62.5μ) on a dry weight basis. At the planted areas of the finger-fill canals, sediments averaged 98.6% sand and 1.4% silt and clay. No analysis of the carbonate fraction was made for these samples; however, all sites had shell fragments, which appeared to be more abundant in the canals than at the control site.

MATERIAL AND METHODS

The work was divided into two phases: Phase I extended from July 1966 through August 1967 and phase II from April through October 1967. In phase I, methods of deflecting and reducing the force of tidal currents and waves in the vicinity of transplants and of anchoring new transplants in the substrate were tested. Concrete building blocks were laid in parallel rows at both transplant sites to form enclosed areas for sheltering new transplants against the forces of moving water (Figure 3). Plugs of grass approximately 8 inches square (20 × 20 cm)

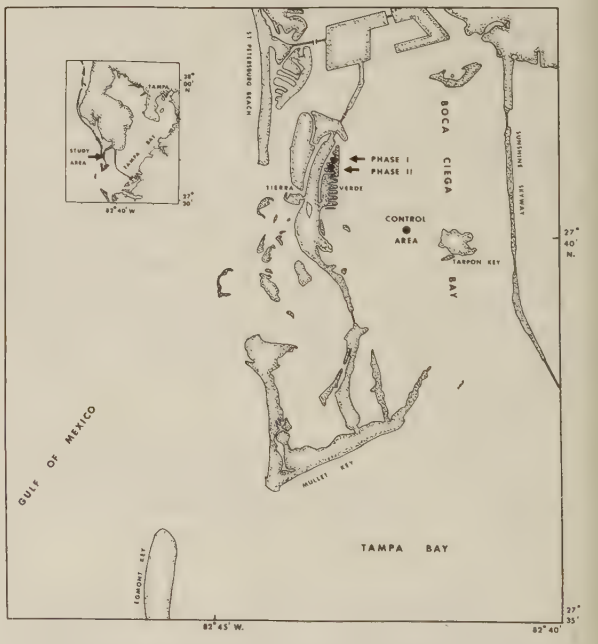

FIGURE 2.—Locations of experiments (phases I and II, and control area).

and containing four to five short-shoots were dug from natural beds adjacent to the control site. Three methods of transporting and anchoring the plugs were tried: (1) placing them in tin cans, (2) balling them in burlap, and (3) temporarily bagging the roots and rhizomes in polyethylene, which was removed just before planting.

A total of 120 plugs was transplanted — 60 at the control site and 60 at the finger-fill canal. At both locations, 30 were placed inside and 30 outside of enclosures. Each group of 30 plugs was planted three ways: 10 in cans, 10 in burlap, and 10 unanchored (Figure 3).

Phase II consisted of testing additional anchoring devices to hold individual sprigs of turtle grass in the substrate of the finger-fill canal. The devices were cast iron, 2-inch (5.1-cm) pipe, brick, and construction rod. Sprigs used in this study were single short-shoots with leaves, many roots, and with or without a portion of the parent rhizome. Also tested in phase II was the plant hormone, NAPH (Naphthalene Acetic Acid),[6] which is used for rooting grass stolons and plant cuttings.

Sixty sprigs, obtained from the same natural bed as the plugs in phase I, were washed and prepared for the experiment by breaking entire rhizomes from some, breaking only the apexes of rhizomes from others, and leaving the rhizomes attached and entire on others. Half of the sprigs were placed in a 10% solution of NAPH in seawater for 1 hr. The other half were left untreated. The sprigs were planted in groups to test various combinations of treatment and nontreatment with NAPH, presence and absence of apexes of rhizomes, presence and absence of rhizomes, and types of anchors (Figure 4).

Sprigs anchored with construction rod had no rhizomes. Sprigs anchored with pipe had rhizomes that were buried in hand-dug holes; whereas, sprigs anchored with brick were simply placed on the surface of the substrate and their rhizomes held in contact with the sediment by the weight of the brick.

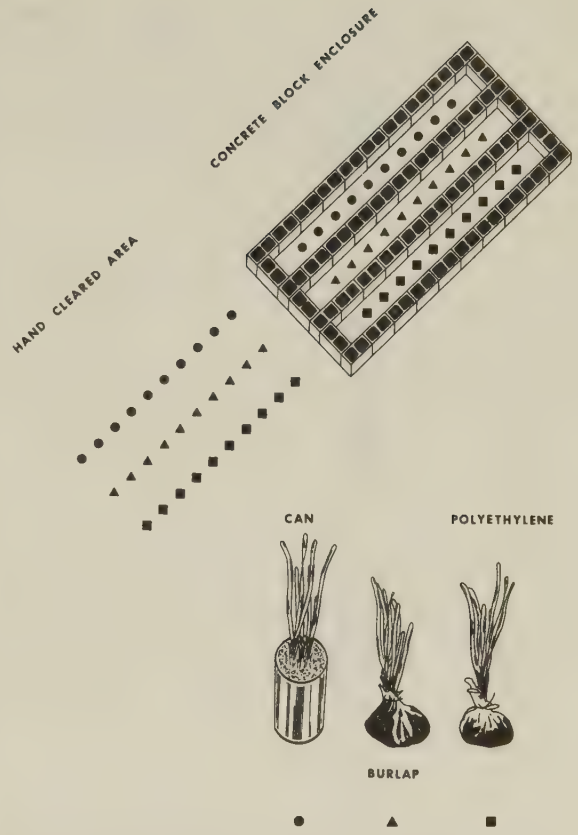

FIGURE 3.—Details of concrete-block wave and current barriers, tin-can anchors for plugs, and placement of transplants at planting sites.

RESULTS

Transplants were considered successful if they established themselves in the new environment and exhibited new rhizome growth (Figure 5). Individual sprigs met these criteria if short-shoots appeared healthy, had new roots, and had either given rise to a new rhizome or were still part of an old long-shoot with an active apex. Plug transplants (phase I) were considered successful if only one of the short-shoots met the above criteria.

[6] Manufactured by Nutri-Sol Chemical Company, Tampa, Fla. 33609. References to trade names in this publication do not imply endorsement of commercial products.

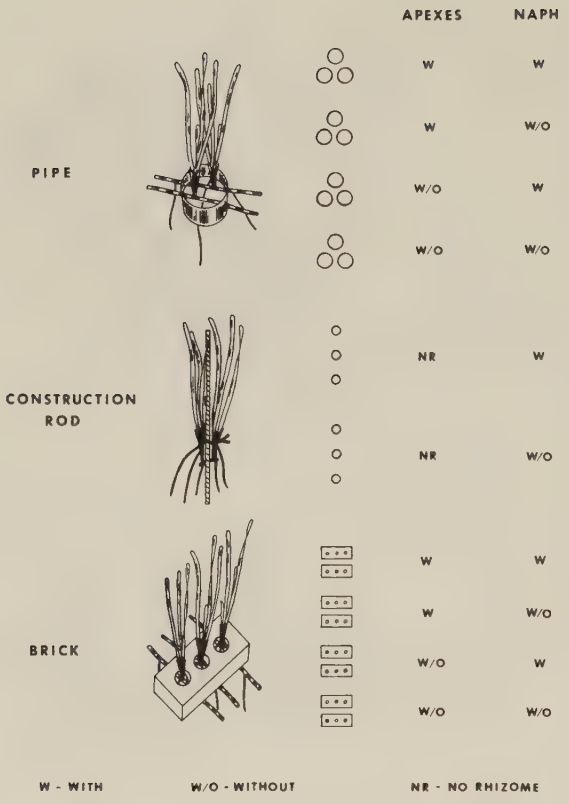

FIGURE 4.—Details and treatment of sprigs anchored by pipe, brick, and construction rod; and placement of transplants at planting sites.

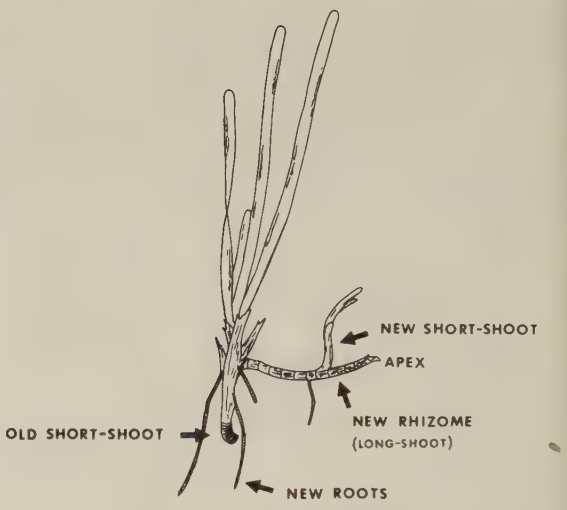

FIGURE 5.—New rhizome, root, and short-root growth on a sprig of turtle grass transplanted without an intact rhizome apex.

Plugs in phase I planted July 1966 were removed from the sites late in August 1967, approximately 13 months after planting. Six of the 40 transplants at the canal area and 16 of the 40 at the control area were successful (Table 1).

Planting individual sprigs of turtle grass in phase II yielded similar results. Of the 60 sprigs planted in the second canal in April 1967 and removed in mid-October 1967 (about 6 months after they were transplanted), 11 were successful (Table 2).

Successful new growth of rhizomes represented 15 and 18% of the number of transplants made in the finger-fill canal in phases I and II, respectively, and 40% of the transplants made in the control bed (Tables 1 and 2). Transplant attempts made with burlap in phase I were not included in the above percentages because all failed within 1 month.

EROSION CONTROL

Results of planting plugs within concrete-block enclosures were completely different in the finger-fill canal and grass bed locations (Table 1). In the canal the only successful transplants grew within the protection of the block enclosures; none planted without this protection survived, and most of the latter failed within the first 6 months of the experiment. Most of the successful plugs placed in the control area were planted outside the concrete blocks and, throughout the study, appeared to be in better condition than those inside the enclosures. The enclosures fulfilled their purpose in the canal but appeared detrimental in the control area. In the latter region, surrounding grass beds apparently provided sufficient protection from water movement. Enclosures in

TABLE 1.—Surviving transplants, successful transplants, and seasonal mortality of transplanted plugs of *Thalassia* in the finger-fill canal and the control site, phase I, July 1966 through August 1967.

Method of protecting and anchoring	Transplants	Surviving plants			Mortality			
		Unsuccessful[1]	Successful[2]		Fall	Winter	Spring	Summer
	No.	*No.*	*No.*	*%*	*No.*	*No.*	*No.*	*No.*
			Finger-fill canal site					
Inside concrete block enclosures:								
Anchored in cans	10	0	3	30	3	4	0	0
Unanchored	10	0	3	30	2	4	0	1
Total	20	0	6	30	5	8	0	1
Outside concrete block enclosures:								
Anchored in cans	10	0	0	0	9	1	0	0
Unanchored	10	0	0	0	10	0	0	0
Total	20	0	0	0	19	1	0	0
Grand total	40	0	6	15	24	9	0	1
			Control site					
Inside concrete block enclosures:								
Anchored in cans	10	1	3	30	5	1	0	0
Unanchored	10	2	0	0	4	1	2	1
Total	20	3	3	15	9	2	2	1
Outside concrete block enclosures:								
Anchored in cans	10	1	7	70	0	1	0	1
Unanchored	10	2	6	60	1	1	0	0
Total	20	3	13	65	1	2	0	1
Grand total	40	6	16	40	10	4	2	2

[1] Transplants survived but did not exhibit new rhizome growth.
[2] Transplants exhibited new rhizome growth.

the control area often filled with a heavy accumulation of light-robbing algae, dead grass, and other detritus, which quickly resulted in burial and death of the entire transplant.

ANCHORING METHODS FOR PLUGS

Of the plugs anchored with tin cans, 50% were successful at the control site, but only 15% in the canal (Table 1). None of the plugs planted in cans outside of the concrete-block enclosures survived. Cans were thus ineffective against currents unless used in conjunction with the concrete-block current barrier.

Plugs transported in polyethylene bags and then directly transplanted served to evaluate the effect of tin cans. We noted no adverse effects from the metal in the cans. The ratio of successes of unanchored to anchored transplants was 3:5.

The reasons for the rapid failure of plugs with roots and rhizomes wrapped in burlap is unknown. Possibly decomposition products of the decaying burlap, such as H_2S, or toxic chemicals in the material caused the plants to die.

ANCHORING METHODS FOR SPRIGS

In phase II sprigs were planted with added anchoring devices but without the aid of the wave and current barriers.

Construction rod was the most effective device used to anchor sprigs. It was the easiest to handle because all sprigs fixed to it were transplanted without rhizomes and were simply fastened to the rod with plastic-coated wire and inserted into hand-dug holes in the substrate. Of the 12 sprigs anchored with rod, only the 6 that had been treated with the hormone NAPH became established (Table 2). Sprigs that did not survive failed early in the experiment and simply disappeared, probably because they were dislodged by water movement in the canal before roots were developed.

TABLE 2.—Surviving transplants, successful transplants, and monthly mortality of transplanted sprigs of *Thalassia* in a finger-fill canal, phase II, April through October 1967.

Method of anchoring and treating	Transplants	Surviving plants			Mortality[1]				
		Unsuccessful[2]	Successful[3]		May	July	Aug.	Sept.	Oct.
	No.	*No.*	*No.*	*%*	*No.*	*No.*	*No.*	*No.*	*No.*
Pipe[4]									
With apexes, with NAPH	6	0	0	0	6	0	0	0	0
With apexes, without NAPH	6	0	0	0	0	6	0	0	0
Without apexes, with NAPH	6	2	2	33.3	0	0	0	2	0
Without apexes, without NAPH	6	5	1	16.7	0	0	0	0	0
Total	24	7	3	12.5	6	6	0	2	0
Brick[5]									
With apexes, with NAPH	6	4	2	33.3	0	0	0	0	0
With apexes, without NAPH	6	0	0	0	0	6	0	0	0
Without apexes, with NAPH	6	0	0	0	0	6	0	0	0
Without apexes, without NAPH	6	0	0	0	3	3	0	0	0
Total	24	4	2	8.3	3	15	0	0	0
Construction rod[6]									
With NAPH	6	0	6	100.0	0	0	0	0	0
Without NAPH	6	0	0	0	0	4	2	0	0
Total	12	0	6	50.0	0	4	2	2	0
Grand total	60	11	11	18.3	9	25	2	2	0

[1] Mortality not observed in June.
[2] Transplants survived but did not exhibit new rhizome growth.
[3] Transplants exhibited new rhizome growth.
[4] Rhizomes were buried; two sprigs per anchor.
[5] Rhizomes were not buried; three sprigs per anchor.
[6] Rhizomes were removed before planting; two sprigs per anchor.

Pipe and brick were poor anchors. The sprigs anchored with pipe were transplanted with their rhizomes and special care was required in burying them to avoid breakage. Almost half of the 24 sprigs held with pipe lived to the end of the experiment, but only 3 exhibited new rhizome growth (Table 2). Sprigs secured to the bottom with brick were not buried but were simply laid on the bottom and the substrate was scooped over them by hand. They were also transplanted with their rhizomes but were difficult to handle because of their tendency to slip out from underneath the brick before they were finally set in place. Six of the 24 sprigs lived for awhile, but only 2 were successful. Sprigs that were anchored with brick and failed did so shortly after they were planted. Water movement probably eroded away enough sediment to allow the buoyant sprigs to float from under the brick.

TREATMENT OF TRANSPLANTS IN PHASE II

The effect of NAPH on marine grasses is apparently similar to its effect on terrestrial plants, primarily inducing rapid and heavy rooting. Ten of the 11 sprigs producing new rhizomic growth were treated with it (Table 2). Because of the small number of transplants attempted and successes achieved, we cannot definitely establish the significance of NAPH in such experiments. Our results indicate to us, however, that NAPH was one of the main factors contributing to transplant success.

Particular care was taken to avoid damaging rhizomes and rhizome apexes of sprigs before and during transplanting. No apparent advantage was gained from this care; invariably old rhizomes withered away and were replaced by new ones developing from the bases of the shortshoots.

MORTALITY OF TRANSPLANTS

Visual checks made throughout the year showed that the most critical period for the survival of turtle grass was during the first 3 months after transplanting. In phase I, mortality of plugs planted in the canal was 60% through the third month (October), 22.5% through the sixth month (January), zero

through the 3-month period February-April, and 2.5% during the remainder of the study. Losses in the control area for the same time intervals were 25, 10, 5, and 5%, respectively.

Mortality experienced during phase II was also high. Over half (57%) of the sprigs transplanted in April failed before the end of the third month (July) and 7% from August to October. Additional failures within this phase might have occurred had the experiment continued through the winter.

CONCLUSIONS AND RECOMMENDATIONS

Our experiments resulted in the first successful field transplantation of turtle grass. All new short-shoots produced by transplants were from the new rhizome apexes (Figure 5). This finding supports the observations of Phillips (1960) and Tomlinson and Vargo (1966) that buds on the rhizome apex are the only source of short-shoots. It is also in agreement with findings in the tank culture of *Thalassia* (Fuss and Kelly, 1969). Continuous growth of turtle grass depends on the activity of vigorous rhizome apexes, but the apexes do not contain the only meristematic tissue in the plant. New rhizomes can be produced from residual meristematic tissue present in the old short-shoot. Phillips (1960) observed such branching in the field and stated that it could account for the continued growth of turtle grass if the apex of the rhizome were damaged or lost, but believed that the frequency of this branching was small. Tomlinson and Vargo (1966) also reported that vegetative branching in short-shoots occurs and indicated that it is rare.

Undamaged leaves may not be required for sprig transplanting. Further studies are needed to determine, for example, if the leaves could be cut back to reduce the surface area and buoyancy of the sprig. Results of investigations in Boca Ciega Bay by Prest, Saloman, and Taylor[7] show that turtle grass leaves clipped as much as 50% of their original height (about 26 cm) would regrow as much as 3 to 4 cm (1.2 to 1.6 inches) per week. It would thus appear that physical damage to leaves is quickly overcome by regrowth of the plant.

We have shown that turtle grass can be transplanted in the field and that it will grow in an area denuded by coastal dredging. A simple transplant method using only the short-shoots of this grass, the hormone NAPH, and construction rod was 100% successful (six transplants) in a land-fill finger canal (Table 2). This method has value for use in restoring *Thalassia* to estuarine environments when conditions favorable for plant growth exist or can be artificially created. We must emphasize however, that no large-scale transplant program has been attempted. Moreover, recent observations (November 1970)[8] of vegetative growth into our original control site indicate that turtle grass spreads at an annual rate of only 20 cm (8 inches) or less.

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EFFECT OF DIETARY FISH OIL ON THE FATTY ACID COMPOSITION AND PALATABILITY OF PIG TISSUES[1]

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ABSTRACT

Basically, this report deals with the problem of a "fishy" flavor in the meat of pigs, which sometimes results when pigs are fed fishery products, such as fish meal, above a certain concentration in the diet.

In this study, pigs were fed diets containing fish oil to investigate specifically: (1) the effect, on the taste of the meat, of feeding pigs fish oil, (2) the effect, on the taste of the meat, of withdrawing the oil from the diet at given times, (3) the fatty acid composition of the various body tissues of the pigs, and (4) the relation of composition to the taste of the meat.

The principal findings of the study were: (1) The amount of the fish oil $\omega 3$ fatty acids fed and deposited was significantly positively correlated with the weighted organoleptic score[4] when the pigs were fed the oil containing diets to a market weight of 90.9 kg. (2) Removal of the fish oil from the pigs' diets when the pigs obtained body weight (of either 68.0 or 79.5 kg) resulted in a loss of the significant positive correlation above. (3) Differences in the degree of unsaturation and in fatty acid composition were found among the oils in the tissues examined. (4) A signifiant positive correlation was obtained between the quantity of the characteristic fatty acids ($\omega 3$) of fish oil fed and the quantity deposited in three of the four tissues examined, the exception being the *longissimus dorsi* tissue.

Both the processors of fishery industrial products and the feed manufacturers who use the products are sometimes confronted with the problem of a fishy flavor in the carcasses of animals fed diets in which these products are included. Fish oil fed directly to the animals or fed as a residual component of fish meal or of fish solubles has been shown to produce an off-flavor under certain conditions (Banks and Hilditch, 1932; Hilditch and Williams, 1964).

Through practical research, the problem has been partly solved by reducing the quantity (that is, the percentage) of fish oil in the diet or by eliminating the oil during an interval of time before the animals are marketed (Frazer, Stothart, and Gutteridge, 1934). This latter technique is not always effective, especially when fairly high (8.25%) levels of fish oil have been fed (Anglemier and Oldfield, 1957).

Investigations to relate more specifically the causal agents of the off flavor resulting from the use of fish oil have led to the hypothesis that the long-chain polyunsaturated fatty acids of the C_{20-22} series commonly found in fish oil are precursors of the flavor-producing components (Banks and Hilditch, 1932; Marion and Woodroof, 1963; Miller, Gruger, Leong, and Knobl, 1967). Investigations by the Animal Nutrition Unit of the Bureau of Commercial Fisheries (now the National Marine Fisheries Service) Technological Laboratory, College Park, Md., using chickens, have indicated that a further partitioning of the C_{20-22} fatty acid series results in a positive correlation between individual fatty acids of these series deposited and the detection of the off-flavor (Miller et al., 1967).

In a continuation of this line of investigation the work reported here was divided into four experiments. Their purposes were to determine the following information:

1. The relation between the menhaden-oil fatty acid fed and the fatty acid pattern of tissue samples (namely, those of the outer and the in-

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[4] Note the organoleptic score increased with greater unacceptability.

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ner backfat, the *longissimus dorsi* muscle, and the liver) of pigs fed various diets with and without menhaden oil and for various intervals of time before they are marketed.

2. The organoleptic effect of the different dietary levels of menhaden oil on the meat of the pigs and the retention or disappearance of the off-flavor by removal of menhaden oil from the diet of the pigs when they reach a body weight of 68.0 or 79.5 kg and are subsequently marketed when they reach a weight of 90.9 kg.

3. The relation, if any, between the detection of off-flavor and the pattern of fatty acid deposition in the tissue samples.

4. The metabolic interrelation of fatty acids of the various fatty acids of the omega families ($\omega 3$, $\omega 6$, $\omega 9$).

RELATION BETWEEN MENHADEN OIL FATTY ACIDS FED TO PIGS AND DEPOSITIONAL PATTERNS OF THESE FATTY ACIDS IN THE PIG TISSUES

Callow (1935, 1938) indicated that the rate of deposition of fat in pigs is correlated with the iodine number of the fat and that slower growing pigs deposit a more unsaturated fat. Accordingly, we felt that our experimental pigs should be handled so that they would develop uniformly, thus minimizing the variation in the composition of depot fat resulting from differential rates of growth.

The first part of this experiment was a general study to monitor the uniformity of growth of the pigs and of the development of their carcasses. That is, we wanted to determine whether the diets fed and our treatment of the pigs would result in any abnormalities that might invalidate the specific findings in this first experiment and in the other three experiments to follow.

UNIFORMITY OF GROWTH OF PIGS AND OF DEVELOPMENT OF CARCASSES

Uniformity of Growth

Described here are the diets, the allotment and management of the pigs, and the statistical analyses used.

The diets were balanced on an equal-protein and equal-calorie basis and were fortified to supply all the known nutrients required by pigs. Crude menhaden fish oil that had been stabilized with butylated hydroxy toluene[5] was added at levels of 0.4% to 1.4%. The oil replaced various proportions of cerelose and Solka Flox[6] to give isocaloric and isonitrogenous diets (Table 1). The diets were mixed in a ribbon-type mixer and were pelleted weekly through a 12-mm die. Steam was not used in the pelleting process. Table 2 shows the gas-liquid chromatographic analyses of the oil and of the diets fed.

[5] Level of addition is trade secret.
[6] Trade names are used merely to simplify descriptions; no endorsement is implied.

TABLE 1.—Diet formulation used in experiment to determine the dietary level of menhaden oil that will impart off-flavors to the meat of pigs.

Ingredients	Concentration of the given ingredients in the diet when the percentage of menhaden oil in the diet was:						
	0	0.4	0.6	0.8	1.0	1.2	1.4
	%	%	%	%	%	%	%
Fixed basal ingredients:							
Corn US #2	67.0	67.0	67.0	67.0	67.0	67.0	67.0
Soybean oil meal	20.3	20.3	20.3	20.3	20.3	20.3	20.3
Alfalfa leaf meal	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Dicalcium phosphate	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Salt (trace mineral)[1]	.6	.6	.6	.6	.6	.6	.6
Vitamin mix[1]	.2	.2	.2	.2	.2	.2	.2
Variable ingredients:							
Cerelose	6.9	5.5	4.8	4.1	3.4	2.7	2.0
Cellulose	--	1.0	1.5	2.0	2.5	3.0	3.5
Menhaden oil	--	.4	.6	.8	1.0	1.2	1.4

[1] Sufficient trace minerals and vitamins were present to meet the requirements of the National Research Council.

TABLE 2.—Gas chromatographic analysis of methyl esters of the fatty acid components of the menhaden oil and of the diets fed to pigs.

Fatty acid	Concentration of the given fatty acid in menhaden oil	Concentration of the given fatty acid in the diet when the percentage of menhaden oil in the diet was:						
		0	0.4	0.6	0.8	1.0	1.2	1.4
	%	%	%	%	%	%	%	%
[1]14:0	5.96	0.17	1.03	1.42	1.62	1.98	2.05	2.51
14:1	--	0.05	0.05	0.06	0.06	0.08	0.08	0.10
15:0	0.34	0.13	0.05	0.15	0.21	0.06	0.06	0.07
?	[3]tr	0.06	0.17	0.05	0.06	0.27	0.27	0.31
15:1	0.09	--	tr	tr	tr	tr	tr	tr
16:0	13.10	11.77	13.15	13.59	13.26	14.10	13.46	14.85
[2]16:1 ω7	10.36	0.31	1.31	1.59	1.86	2.31	2.34	2.85
17:0	0.64	0.16	0.21	0.24	0.26	0.26	0.27	0.31
?	--	0.11	tr	tr	tr	tr	tr	tr
16:2	--	--	--	--	--	--	--	--
17:1	1.06	0.10	0.23	0.29	0.32	0.36	0.39	0.45
?	0.10	--	0.04	0.04	--	0.03	0.05	0.05
18:0	4.36	2.70	2.86	2.95	3.17	3.16	3.26	3.17
18:1 ω9	27.59	27.45	24.97	24.06	23.57	22.90	22.63	20.44
19:0	1.45	0.10	0.27	0.35	0.42	0.43	0.48	0.58
18:2 ω6	1.57	51.22	44.60	41.86	40.96	39.14	38.22	35.22
?	0.32	tr	tr	tr	tr	tr	tr	tr
?	0.24	0.11	0.13	0.14	0.18	0.12	0.16	0.17
20:0	0.31	0.78	0.78	0.75	0.81	0.65	0.68	0.69
18:3 ω3	0.94	3.31	2.60	2.89	2.75	2.62	2.49	2.97
20:1	1.32	0.40	0.68	0.73	0.76	0.75	0.79	0.86
18:4 ω3	2.89	--	0.50	0.65	0.76	0.84	0.94	1.11
?	0.37	0.07	0.17	0.19	0.24	0.21	0.27	0.30
20:2 ω9	0.18	0.07	0.11	0.12	0.13	0.07	0.13	0.14
20:2 ω6	0.06	tr	tr	tr	tr	tr	tr	0.06
20:3 ω9	0.13	tr	0.08	0.08	0.09	0.05	0.10	0.11
22:3 ω6	0.05	tr	tr	0.04	tr	tr	tr	tr
20:4 ω6	0.69	0.22	0.37	0.42	0.42	0.37	0.45	0.53
22:1 ω2	0.30	tr	0.12	0.09	0.12	0.07	0.13	0.16
20:4 ω3	1.25	0.08	0.29	0.34	0.41	0.42	0.52	0.59
20:5 ω3	12.95	0.11	2.19	2.95	3.35	3.96	4.28	5.01
?	--	0.23	0.40	0.26	0.34	0.15	0.28	0.40
24:0	0.09	--	tr	tr	tr	tr	tr	tr
22:4 ω6	0.59	0.29	0.53	0.47	0.48	0.46	0.54	0.57
22:5 ω6	0.43	--	tr	0.24	tr	0.26	0.46	0.45
22:5 ω3	1.63	--	0.40	0.50	0.63	0.65	0.77	0.86
22:6 ω3	8.47	--	1.71	2.49	2.76	3.29	3.44	4.14

[1] "14:0" means that the fatty acid has 14 carbon atoms per molecule and no unsaturated bond.
[2] "16:1 ω7" means that in the fatty acid the unsaturated bond occurs at the seventh bond from the terminal methyl group.
[3] "tr" means trace.

Seven Yorkshire gilts each weighing about 27.3 kg were allotted to each of the seven treatment groups. Two of the seven pigs of each menhaden-oil group were fed the appropriate oil-containing diet until they attained a body weight of 68.0 kg and then were fed the control diet until they attained a body weight of 90.9 kg. Similarly, two additional pigs of each menhaden-oil group were fed the appropriate oil-containing diet to a body weight of 79.5 kg and then also were fed the control diet to a body weight of 90.9 kg. The remaining three pigs were continuously fed the various test diets containing menhaden oil until they each also attained a body weight of 90.9 kg. Feed was offered twice a day (for a maximum of 1 hr per feeding) to the pigs in individual crate-type pens. This interval of time was considered to be adequate to permit the pigs to eat the same total amount of food that they would have eaten ad lib. Data on rates of gain and consumption of feed were recorded weekly.

Data obtained on rates of gain and utilization of feed were subjected to an analysis of variance (Snedecor, 1956).

Table 3 presents the rates of gain, utilization of feed, and quantity of oil consumed by the pigs fed diets containing the various percentages of menhaden oil.

Results of the analyses of variance for each

TABLE 3.—Rates of gain, utilization of feed, and quantity of oil consumed by pigs fed diets containing various percentages of menhaden oil.

Relative amount of menhaden oil in diet	Average daily gain		Ratio of feed to gain		Mean quantity of oil consumed by pigs to a body weight of:		
	Mean	SD	Mean	SD	68.0 kg	79.5 kg	90.9 kg
%	kg	kg			kg	kg	kg
0	0.63	0.065	3.45	0.136	0	0	0
0.4	.64	.065	3.26	.105	0.52	0.62	0.85
0.6	.60	.047	3.34	.093	0.85	0.96	1.30
0.8	.64	.045	3.47	.095	1.03	1.32	1.90
1.0	.64	.025	3.26	.080	1.20	1.48	2.16
1.2	.66	.068	3.28	.100	1.53	1.78	2.70
1.4	.64	.044	3.25	.084	1.61	2.12	3.22

criterion of evaluation indicate that these criteria did not differ significantly.

Development of Carcasses

The yield of lean cuts was obtained as an accumulative value for the four commercial lean cuts—namely, hams, loins, shoulders (picnics), and Boston butts. Cross-sectional measurements of the *longissimus dorsi* muscle of the loin were obtained by cutting the loin at the 10th rib, tracing the muscle area onto paper, and measuring the perimeter of the area by means of a planimeter to convert the encompassed area to square centimeters. The thickness of the backfat was based on an average of three measurements taken at positions opposite the first rib, the last rib, and the last lumbar vertebra.

Table 4 presents the data on the dressing percentage, lean-cut percentage, *longissimus dorsi* area, and backfat thickness obtained from pigs fed the various diets containing menhaden oil. The analyses of variance for each criterion of evaluation indicate that no significant differences occurred among these factors that reveal the growth reaction of the pigs to their diet.

Thus the pigs developed uniformly during the feeding trials. Consequently any differences that may be found in the fatty acid composition of the tissues should be related to the oil in the diet rather than to markedly different growth of the pigs.

RELATION OF DEPOSITIONAL PATTERNS TO FATTY ACIDS IN OIL FED TO PIGS

In this section, we are concerned with the following three subjects: (1) the differences found in the degree of saturation both within and among tissues, (2) the fatty acids identified, and (3) the relations of the quantity of fatty acids fed to the quantity deposited in the various tissues.

Differences Found in Degree of Saturation Both Within and Among Tissues

Described here are (1) the tissue samples used, (2) the extraction of lipids, (3) the preparation of methyl esters, and (4) the quantitative gas-liquid-chromatographic technique.

Samples were taken from the outer and the inner backfat tissue, the *longissimus dorsi*, and the liver in the following manner. From each animal, a sample of backfat was obtained dorsally to the 10th to 12th ribs. This sample was then divided into the "outer" and "inner" fat layers. Samples of the muscle were taken from the eye of the *longissimus dorsi* at the 10th rib. Samples of the liver were taken from the right central lobe. All samples were placed in vials, protected with nitrogen, and held at —20° C until the lipids were extracted from them.

The lipids were extracted from the samples by the homogenization of the tissue in a mechanical blender with a 2:1 mixture of chloroform and methanol for 2 min. The solvent mixture was added in the proportion of 5 ml of mixture to 1 g of sample. The slurry was filtered through a Buchner funnel, and the filter paper and the nonfilterable portion were re-extracted for another 2-min period. The filtrate was evaporated in a rotary vacuum evaporator over a 60° C water bath. The dried sample was redissolved in

TABLE 4.—Dressing percentage, lean-cut percentage, *longissimus dorsi* area, and backfat thickness obtained from pigs fed various diets containing menhaden oil.

Relative amount of menhaden oil in diet	Relative yield of:				*Longissimus dorsi* area		Backfat thickness	
	Dressing		Lean cuts[1]					
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
%	%	%	%	%	cm^2	cm^2	*cm*	*cm*
0	83.8	±2.36	38.9	±2.45	32.39	±5.78	3.56	±0.62
0.4	83.4	±1.23	40.2	±1.68	33.68	±4.83	3.30	± .45
0.6	82.0	±1.89	39.6	±1.07	31.87	±3.68	3.61	± .19
0.8	83.1	±1.06	39.4	±1.32	30.78	±5.08	3.61	± .29
1.0	82.7	±1.62	40.1	±0.88	32.78	±4.39	3.30	± .27
1.2	82.0	±2.21	38.3	±2.14	31.74	±4.00	3.53	± .62
1.4	82.1	±1.86	38.1	±1.61	30.91	±5.19	3.65	± .40

[1] Calculated as the sum of weights of Boston butts, shoulder, loin and ham, as a percentage of weight of dressed carcass.

petroleum ether (30° to 60° C boiling point), poured into a separatory funnel, and washed twice with a 20% solution of NaCl. The layer of petroleum ether was evaporated in the rotary evaporator, and the extracted fat was transferred to containers in which it was protected by nitrogen and was stored at —20° C until methyl esters were prepared from it for analysis.

The methyl esters of the fatty acids were prepared as follows:

Five ml of anhydrous methanol and about 50 mg of freshly cut and shiny sodium were placed into a small test tube. After the sodium had reacted, six to eight drops of the extracted oil were added and heated to reflux on a steam bath for 2 min with agitation. The end point of the reaction was signaled when the solution became clear.

The reaction solution was quenched with 5 ml of distilled water and was transferred to a separatory funnel. The mixture was extracted with two 10-ml portions of petroleum ether (30° to 60° C boiling point). The final water layer was discarded, and the two petroleum ether extracts were combined. The petroleum ether solution was washed with 10 ml of 5% aqueous HCl solution. The acid wash was followed by successive washes with 15-ml and 10-ml aliquots of 20% NaCl solution. The washing was completed when pH paper tested neutral.

The ethereal solution of methyl esters was dried over 3 g of anhydrous Na_2SO_4, filtered, and evaporated over a 60° C water bath, using a vacuum rotary evaporator.

To check for purity, we made a thin-layer chromatogram of the ester solution using silicic acid paper. Methyl myristate was used as the control. A solution of 90 parts petroleum ether, 10 parts ethyl ether, and 1 part formic acid was used to elute the esters. The chromatogram was developed in iodine vapor.

Methyl esters of pure fatty acids were used as reference standards for the C_{14-24} saturated acids, C_{16-24} monoenoic acids, plus linoleic, linolenic, arachidonic, eicosapentaenoic, and docosahexaenoic acids. Also concentrates of 16:2, 16:3, 16:4, and 18:4 methyl esters that were obtained by fractional distillation and urea-inclusion compound fractionalization were used as reference standards.[7] As a secondary reference mixture, methyl esters from whole menhaden oil were also analyzed. From a plot of the logarithms of the retention times (relative to stearate) versus the number of carbon atoms, nearly linear relations were observed for homologous series (Farquhar, Insull, Rosen, Stoffel, and Ahrens, 1959). Identifications were further verified by applying the graphical method of James (1960) for analyses on columns packed with diethylene glycol succinate polyester and Apiezon L. These plots provided the necessary reference data for identification of the various tissue lipids analyzed.[8]

[7] The staff of the National Marine Fisheries Service Technological Laboratory, Seattle, Wash., made the fractional distillations and urea-inclusion compound fractionations.

[8] The fatty acids of the oil fed and of the animal tissues were identified initially in collaboration with the staff of the National Marine Fisheries Service Technological Laboratory, Seattle, Wash.

Methyl esters of fatty acids taken from the various tissues were analyzed with an F&M Biomedical Model 400 gas chromatograph. The instrument was equipped with a hydrogen flame detector. The column used was composed of 4.0-mm ID by 243.8-cm Pyrex glass containing 5.0% (by weight) of diethylene glycol succinate polyester (DEGS from Wilkens Instrument and Research Inc.) supported on 80- to 90-mesh acid-base washed and siliconized flux-calcinated diatomaceous earth (Anakron ABS). The operating conditions were as follows: column temperature, 165° C; flash-heater temperature, 285° C; detector temperature, 200° C; and initial attenuation that corresponds to 10 to 14 amp full-scale deflection. The inlet pressure of the column measured 40 psi of helium, the flow measured 53 ml per min at the outlet of the column. The size of the injected sample was about 0.12 μliter.

The area-percent method was used to determine the corresponding peak areas of the curves obtained from the gas-liquid chromatographic recorder. The fatty acid composition (in average percentage) of each sample was calculated by multiplying peak height by retention time and then multiplying this product by 100 and dividing by the total area.

Certain differences were obtained in the total degree of saturation and quantity of specific

TABLE 5.—Summary of gas-liquid chromatographic analyses indicating comparative degree of unsaturation and quantity of selected fatty acids within and among the tissues obtained from pigs fed either 0% or 1.4% dietary menhaden oil.

Type of fatty acid	Concentration of the various fatty acids in the various tissues when the relative amount of menhaden oil in the diets was:							
	0%				1.4%			
	Backfat				Backfat			
	Inner tissue	Outer tissue	*Longissimus dorsi* tissue	Liver tissue	Inner tissue	Outer tissue	*Longissimus dorsi* tissue	Liver tissue
	%	%	%	%	%	%	%	%
Saturated fatty acids	34.56	28.37	33.74	34.63	35.42	28.77	33.05	34.66
Unsaturated fatty acids	65.44	71.63	62.82	65.37	64.58	71.23	64.62	65.34
Unsaturated bond in the fatty acids:								
1	47.68	49.03	39.38	17.53	47.17	46.16	45.96	15.44
2	16.35	20.49	14.40	16.85	17.39	20.75	11.46	17.43
3	0.93	1.40	1.37	2.35	1.40	1.73	1.15	1.85
4	0.40	0.72	4.95	19.08	0.84	0.88	3.27	12.80
5	0.19	0.27	2.03	2.96	0.80	1.53	2.17	8.52
6	0.03	0.05	0.69	2.26	0.40	0.50	0.62	8.11
Equivalent degree of unsaturation in the fatty acids:								
1	47.68	49.03	39.38	17.53	47.17	46.16	45.96	15.44
2	32.70	40.98	28.80	33.70	34.78	41.50	22.92	34.86
3	2.79	4.20	4.11	7.05	4.20	5.19	3.45	5.55
4	1.60	2.88	19.80	76.32	3.36	3.52	13.08	51.20
5	0.95	1.35	10.15	14.80	4.00	7.65	10.85	42.60
6	0.18	0.30	4.14	13.56	2.40	3.00	3.75	48.66
Total	85.90	98.74	106.38	162.96	95.91	107.02	99.98	198.31
Individually selected fatty acids:								
16:0	19.90	18.49	21.78	13.04	20.39	18.64	19.62	12.80
18:0	12.18	7.78	9.24	19.37	12.49	8.08	10.74	18.76
18:1 ω9	43.67	44.94	34.33	15.86	40.52	42.04	41.16	13.57
18:2 ω6	15.07	18.99	13.24	15.27	16.18	19.40	10.47	15.87
18:3 ω3	0.74	1.18	0.59	0.44	1.02	1.37	0.52	0.57
18:4 ω6	0.34	0.49	2.44	18.29	0.32	0.41	1.61	12.15
20:4 ω3	0.06	0.08	2.42	0.49	0.20	0.31	1.61	0.54
20:5 ω3	0.09	0.13	0.98	0.51	0.27	0.52	1.12	4.18
22:5 ω3	0.10	0.14	1.05	2.45	0.53	1.01	1.04	4.34
22:6 ω3	0.03	0.05	0.69	2.26	0.40	0.50	0.62	8.11

Note: The equivalent degree of unsaturation in the fatty acids was obtained by multiplying the number of double bonds by the quantity of fatty acid.

fatty acids found within and among the tissues examined. All the fatty acids that were identified will be discussed in the next section. For illustrative purposes, Table 5 presents selected results obtained with the various tissues. The outer backfat had the lowest total concentration of saturated fatty acids of all the tissues, regardless of whether the diet contained menhaden oil or did not contain it. The remaining tissues (inner backfat, liver, and *longissimus dorsi*) were all higher than the outer backfat and did not differ markedly from each other in the total concentration of saturated fatty acids.

The difference in degree of saturation when confined to comparisons between the inner and outer backfat is in agreement with reports by Banks and Hilditch (1932) and Sink, Watkins, Ziegler, and Miller (1964). The simple ratio of the total quantity of saturated to unsaturated fatty acids, however, does not describe the true character of the unsaturated fatty acids found within the tissues or among them.

An examination of the quantity of unsaturation on the basis of the number of double bonds and the relative quantities of the corresponding fatty acid groups indicates marked differences among the tissues.

Both the *longissimus dorsi* tissue and the liver tissue contain markedly less fatty acids with one unsaturated bond than do either of the backfat tissues, regardless of the dietary treatment. This difference no doubt is reflected by the 18:1 ω9 content.

The concentration of fatty acids with two unsaturated bonds in the outer backfat tissue is higher than that in the remaining tissues and apparently indicates a differential concentration of 18:2 ω6.

The difference most evident among the tissues with respect to the fatty acids with three unsaturated bonds is the higher concentration found in the liver tissue.

Both the *longissimus dorsi* and the liver tissue contained considerably more of the four-unsaturated-bond fatty acids than did the backfat tissues. The liver, in turn, contained about four times the concentration found in the *longissimus dorsi*. Incorporating menhaden oil into the diet lowered the magnitude of these differences among the tissues.

The relative differences among the tissues in the case of the *longissimus dorsi* tissue reflect about equal quantities of the isomeric fatty acids 20:4 ω6 and 20:4 ω3. The concentration of the fatty acids with four unsaturated bonds in the liver tissue is due primarily to the 20:4 ω6 isomer; only small concentrations of the 20:4 ω3 isomer were found.

Similarly, the concentration of fatty acids with five and six unsaturated bonds in the *longissimus dorsi* and liver tissues was markedly higher than in the backfat tissues. The incorporation of menhaden oil into the diet resulted in increased concentrations of these fatty acids in all tissues, although the differences among tissues were of the same magnitude as the differences occurring in the absence of the menhaden oil. The variable concentrations of the fatty acids with five and six unsaturated bonds, owing to treatment differences, reflect differences in the quantities of 20:5 ω3, 22:5 ω3, and 22:6 ω3 fatty acids.

On the basis of the equivalent degree of unsaturation obtained by the multiplication of the number of unsaturated bonds by the quantity of fatty acids of that category, the relative degree of unsaturation of the four tissues is: inner backfat, 85.9; outer backfat, 98.7; *longissimus dorsi*, 106.4; and liver, 164.0. The incorporation of menhaden oil did not change the relative differences among tissues, but it did result in a treatment difference. The relative degree of unsaturation among the treatments was of the magnitude of 10 to 30 units greater for all tissues except the *longissimus dorsi*.

Thus, these results generally conform with those previously reported that various tissues differ in fatty acid composition (Brown and Deck, 1930; Banks and Hilditch, 1932; Sink et al., 1964) and that dietary oils alter this fatty acid pattern and degree of unsaturation of the animal tissues of monogastric animals (Ellis and Isbell, 1926a, 1926b; Ellis and Zeller, 1930; Ellis, Rothwell, and Pool, 1931; Bhattacharya and Hilditch, 1931; Hilditch and Pedelty, 1940).

Fatty Acids Identified

Table 6 reports the fatty acids identified by the method of gas-liquid chromatographic analysis described in the preceding section.

TABLE 6.—Fatty acids identified in pig tissues.

Fatty acid	Presence or absence of the fatty acid in:			
	Backfat		*Longissimus dorsi* tissue	Liver tissue
	Inner tissue	Outer tissue		
22:6 ω3	+	+	+	+
22:5 ω3	+	+	+	+
20:5 ω3	+	+	+	+
20:4 ω3	+	+	+	+
18:4 ω3	+	+	+	+
18:3 ω3	+	+	+	+
22:5 ω6	−	trace	trace	+
22:4 ω6	−	+	+	+
20:4 ω6	+	+	+	+
20:2 ω6	−	−	+	+
18:2 ω6	+	+	+	+
21:1 ω9	−	−	+	+
20:2 ω9	+	+	+	+
20:1 ω9	+	+	+	+
18:1 ω9	+	+	+	+
22:1 (?)	−	−	trace	+
20:3 (?)	+	+	+	+
16:2	+	+	+	+
16:1 ω7	+	+	+	+
15:1	+	+	trace	+
14:1	+	+	+	+
20:0	+	+	+	+
19:0	+	−	+	+
18:0	+	+	+	+
17:0	+	+	+	+
16:0	+	+	+	+
15:0	+	+	+	+
14:0	+	+	+	+

Twenty-eight fatty acids were identified in the liver tissue, whereas a lesser number was identified in the three other tissues (inner and outer backfat and *longissimus dorsi*). The fatty acids identified included those reported by Sink et al. (1964) plus unsaturated 18, 20, 22 carbon fatty acids of three of the fatty acid families —ω3, ω6, and ω9—according to current classification (Mohrhauer and Holman, 1963a).

With respect to the two backfat tissues, the acids found in addition to those reported by Sink et al. (1964) are as follows: 15:1, 16:2. 20:1 ω9, 18:4 ω3, 20:2 ω9, 20:3, 20:4 ω3, 20:5 ω3, 22:4 ω6, and 22:5 ω3. The liver and *longissimus dorsi* tissue also contained 20:2 ω6, 21:1 ω9, 22:1, 22:5 ω6, and 22:6 ω3. Hill (1966) reported, however, the presence of most of these fatty acids in various tissues of miniature pigs with the exception of 20:4 ω3, which we found in our pigs. All of these fatty acids, except 20:4 ω3, have also been noted in rat tissue (Mohrhauer and Holman, 1963a, 1963b, 1963c), and all of them including 20:4 ω3, have also been noted in chick tissue (Miller et al., 1967), in fish tissues and in seal tissue (Ackman, Burgher, and Jangaard, 1963; Ackman, Jangaard, Hoyle, and Brockerhoff, 1964).

The relation of the fatty acids fed (X) to those deposited in the various tissues (Y) was established by correlation and polynomial regression analyses. A polynomial regression computer program prepared by the Biomedical Division of the University of California, Los Angeles, was used. The extent of analysis of the data was limited to the fourth polynomial degree. Regression coefficients, standard errors of regression, correlation coefficients, analyses of variance, and data plots (predicted and observed) were obtained.

Correlation and polynomial regression analyses of the gas-liquid chromatographic data presented in Tables 7 to 10 indicate that the marine-type polyunsaturated fatty acids of the linolenic acid (ω3) family were deposited in all four tissues examined.

In general, a significant positive correlation was obtained between the quantity of the ω3 fatty acids fed and the quantity deposited in the various tissues. This relation was not obtained, however, with the *longissimus dorsi* tissue. The only explanation we have is that the reaction caused by difficulties in the extraction of the fatty acids and their subsequent separation masked any pattern.

Definite relations between the amounts of most of the ω3 fatty acids fed to pigs and the amounts deposited were found in the liver tissues and in the inner backfat tissues and the outer ones.

Specifically, the quantity of two of the menhaden oil fatty acids (22:5 ω3, and 22:6 ω3) found in the liver was positively correlated (0.01%) with the quantity of oil fed to the pigs until they were of market weight (90.9 kg). The correlation for 20:5 ω3 approached significance.

TABLE 7.—Liver tissue: concentration of fatty acids found in liver tissue and correlation to quantity of various fatty acids fed for various time intervals.

Fatty acid	Pig weight group	Concentration of fatty acid in liver tissue when the percentage of menhaden oil in the diet was:							Correlation coefficient	Kind of regression line
		0	0.4	0.6	0.8	1.0	1.2	1.4		
	Kg	*Area percent of fatty acid*								
22:6 ω3	90.9	2.25	5.70	7.88	7.72	8.40	8.90	7.49	0.69**	Quadratic
	79.5	2.25	4.65	4.98	5.11	6.25	5.82	7.43	0.70**	Linear
	68.0	2.25	4.30	4.70	4.33	5.69	5.54	5.12	0.65*	Linear
22:5 ω3	90.9	2.55	3.66	4.42	4.48	5.12	5.17	4.85	0.78**	Quadratic
	79.5	2.55	2.94	3.59	3.47	4.00	3.73	4.05	0.56*	Linear
	68.0	2.55	2.70	4.64	3.75	3.70	3.93	4.11	0.59*	Linear
20:5 ω3	90.9	0.56	3.55	6.23	6.52	8.11	8.46	8.14	0.89**	Quadratic
	79.5	0.56	1.57	2.07	1.96	3.33	2.30	2.20	0.50	--
	68.0	0.56	1.00	1.25	1.65	2.12	3.49	2.20	0.67*	Linear
20:4 ω3	90.9	0.39	0.71	0.54	0.50	0.56	0.43	0.44	−0.21	--
	79.5	0.39	1.45	0.75	0.50	0.60	0.69	0.29	−0.42	--
	68.0	0.39	0.58	0.11	0.67	0.71	0.54	0.89	0.39	--
18:4 ω3	90.9	0.08	0.11	0.05	0.08	0.09	0.12	0.07	0.00	--
	79.5	0.08	0.26	0.05	0.07	0.04	0.06	0.06	−0.29	--
	68.0	0.08	0.07	0.05	0.08	0.06	0.10	0.09	0.33	--
18:3 ω3	90.9	0.47	0.67	0.59	0.66	0.73	0.94	0.66	0.26	--
	79.5	0.47	0.77	0.89	0.54	0.56	0.51	0.48	−0.39	--
	68.0	0.47	0.52	0.45	0.57	0.50	0.39	0.59	0.12	--
22:5 ω6	90.9	0.24	0.05	0.06	0.05	0.12	0.11	0.03	−0.27	--
	79.5	0.24	0.23	0.10	0.05	0.17	0.16	0.00	−0.60*	Linear
	68.0	0.24	0.17	0.10	0.10	0.09	0.14	0.03	−0.83**	Linear
22:4 ω6	90.9	1.21	0.43	0.28	0.33	0.29	0.33	0.21	−0.65**	Cubic
	79.5	1.21	0.53	0.34	0.45	0.47	0.48	0.40	−0.63*	Cubic
	68.0	1.21	0.74	0.74	0.64	0.58	0.53	0.63	−0.69*	Linear
20:4 ω6	90.9	18.58	12.37	11.53	10.50	8.82	8.84	9.69	−0.70**	Quadratic
	79.5	18.58	15.23	14.48	14.65	15.12	14.99	12.84	−0.19	--
	68.0	18.58	17.93	16.95	15.98	16.06	16.66	13.92	−0.74**	Linear
20:2 ω6	90.9	0.52	0.19	0.18	0.20	0.14	0.17	0.17	−0.52*	Cubic
	79.5	0.52	0.43	0.50	0.46	0.26	0.40	0.36	−0.34	--
	68.0	0.52	0.20	0.40	0.23	0.40	0.48	0.38	0.13	--
18:2 ω6	90.9	15.87	17.03	16.17	16.32	15.95	15.93	15.79	−0.33	--
	79.5	15.87	14.28	15.69	16.00	17.05	15.93	15.40	0.16	--
	68.0	15.87	16.78	16.27	16.10	16.39	14.48	16.41	−0.12	--
21:1 ω9	90.9	0.27	0.31	0.13	0.15	0.15	0.15	0.14	−0.59**	Linear
	79.5	0.27	0.52	0.45	0.30	0.13	0.25	0.20	−0.40	--
	68.0	0.27	0.42	0.22	0.28	0.29	0.27	0.18	−0.47	--
20:2 ω9	90.9	0.82	0.54	0.60	0.54	0.59	0.58	0.54	−0.12	--
	79.5	0.82	1.29	0.78	0.68	0.44	0.63	0.52	−0.48	--
	68.0	0.82	0.42	0.59	0.56	1.25	0.59	1.05	0.52	--
20:1 ω9	90.9	0.28	0.24	0.19	0.30	0.20	0.23	0.17	−0.22	--
	79.5	0.28	0.44	0.41	0.26	0.20	0.21	0.22	−0.38	--
	68.0	0.28	0.22	0.27	0.25	0.22	0.23	0.25	−0.28	--
18:1 ω9	90.9	16.61	15.28	13.16	14.80	13.56	14.91	14.17	−0.09	--
	79.5	16.61	16.49	18.55	16.18	12.67	13.33	13.56	−0.49	--
	68.0	16.61	15.64	15.55	16.09	14.59	9.68	12.99	−0.54	--

* $P < .05$ ** $P < .01$

Polynomial regression analyses of these data indicate that the incorporation pattern of the fatty acids (20:5 ω3, 22:5 ω3, 22:6 ω3) was quadratic, with the rate of deposition being greater at the lower levels in the diet. Removal of menhaden oil from the diet of the pigs at the two body weights, 68.0 or 79.5 kg, did not alter this pattern markedly. The principal changes were a reduction in the relative degree of significant response (0.01% to 0.05%) and an alteration

TABLE 8.—Inner backfat tissue: concentration of fatty acids found in backfat tissue and correlation to quantity of various fatty acids fed for various time intervals.

Fatty acid	Pig weight group	Concentration of fatty acid in inner backfat tissue when the percentage of menhaden oil in the diet was:							Correlation coefficient	Kind of regression line
		0	0.4	0.6	0.8	1.0	1.2	1.4		
	Kg	*Area percent of fatty acid*								
22:6 ω3	90.9	0	0.13	0.28	0.29	0.54	0.49	0.48	0.76**	Linear
	79.5	0	0.19	0.21	0.51	0.37	0.31	0.40	0.56*	Linear
	68.0	0	0.09	0.15	0.20	1.06	0.28	0.31	0.49	--
22:5 ω3	90.9	0.10	0.35	0.48	0.54	0.96	0.73	0.88	0.77**	Linear
	79.5	0.10	0.25	0.46	0.28	0.60	0.23	0.19	−0.01	--
	68.0	0.10	0.18	0.20	0.39	0.24	0.54	0.51	0.71**	Linear
20:5 ω3	90.9	0.07	0.12	0.18	0.29	0.44	0.41	0.39	0.71**	Linear
	79.5	0.07	0.08	0.17	0.20	0.27	0.16	0.22	0.55*	Linear
	68.0	0.07	0.04	0.11	0.12	0.12	0.33	0.19	0.71**	Linear
20:4 ω3	90.9	0.06	0.15	0.17	0.24	0.34	0.26	0.29	0.72**	Linear
	79.5	0.06	0.11	0.14	0.23	0.34	0.19	0.17	0.43	--
	68.0	0.06	0.06	0.10	0.19	0.11	0.14	0.14	0.89**	Linear
18:4 ω3	90.9	0.06	0.10	0.13	0.13	0.18	0.16	0.18	0.70**	Linear
	79.5	0.06	0.10	0.11	0.11	0.17	0.06	0.12	0.15	--
	68.0	0.06	0.06	0.04	0.08	0.10	0.15	0.30	0.54	--
18:3 ω3	90.9	0.74	0.82	0.89	0.94	1.04	1.02	1.10	0.52*	Linear
	79.5	0.74	0.72	0.88	0.92	1.08	0.77	0.82	0.17	--
	68.0	0.74	0.86	0.80	0.77	1.03	0.71	1.15	0.52	--
22:5 ω6	90.9									
	79.5				Not identified				--	--
	68.0									
22:4 ω6	90.9									
	79.5				Not identified				--	--
	68.0									
20:4 ω6	90.9	0.34	0.33	0.33	0.29	0.39	0.44	0.33	0.03	--
	79.5	0.34	0.28	0.35	0.38	0.34	0.32	0.33	0.09	--
	68.0	0.34	0.36	0.30	0.34	0.34	0.32	0.28	−0.60*	Linear
20:2 ω6	90.9									
	79.5				Not identified				--	--
	68.0									
18:2 ω6	90.9	15.07	15.53	15.45	15.25	17.51	16.30	17.38	0.41	--
	79.5	15.07	13.15	15.43	16.46	17.97	15.16	14.06	0.10	--
	68.0	15.07	17.45	16.70	14.69	17.48	13.12	17.09	−0.01	--

* $P < .05$ ** $P < .01$

in the patterns of incorporation of these fatty acids (quadratic to linear).

No statistical relation was found for the remaining three fatty acids (18:3 ω3, 18:4 ω3, and 20:4 ω3).

Results of statistical analyses of the data obtained with the inner backfat tissue and the outer backfat tissue, however, indicate that all six of the fatty acids of the ω3 family are deposited in the tissue in proportion to the quantity consumed by the pigs. When menhaden oil was fed to the pigs until they attained a body weight of 90.9 kg, a highly significant positive correlation was obtained for all six ω3 fatty acids. Removing the oil from the diet of the pigs when they weighed 79.5 kg changed the pattern slightly with respect to the outer backfat tissue. The quantity of 18:4 ω3 fed was no longer correlated with the amount deposited. Removal of the oil when the pigs weighed 68.0 kg resulted in only three of the ω3 fatty acids (20:5 ω3, 22:5 ω3, and 22:6 ω3) being correlated.

Similarly with respect to inner backfat tissue, removal of the menhaden oil when the pigs weighed either 68.0 or 79.5 kg resulted in the quantity of certain of the ω3 fatty acids fed no longer being correlated with the quantity deposited. Polynomial regression analyses of

TABLE 9.—Outer backfat tissue: concentration of fatty acids found in outer backfat tissue and correlation to quantity of various fatty acids fed for various time intervals.

Fatty acid	Pig weight group	Concentration of fatty acid in outer backfat tissue when the percentage of menhaden oil in the diet was:							Correlation coefficient	Kind of regression line
		0	0.4	0.6	0.8	1.0	1.2	1.4		
	Kg	— — — — — — — — *Area percent of fatty acid* — — — — — — — —								
22:6 ω3	90.9	0.04	0.23	0.37	0.47	0.59	0.69	0.58	0.85**	Linear
	79.5	0.04	0.25	0.24	0.35	0.63	0.30	0.49	0.58*	Linear
	68.0	0.04	0.20	0.26	0.16	0.34	0.33	0.43	0.74**	Linear
22:5 ω3	90.9	0.14	0.54	0.52	0.85	1.01	1.06	1.14	0.89**	Linear
	79.5	0.14	0.52	0.52	0.66	0.85	0.78	1.04	0.85**	Linear
	68.0	0.14	0.36	0.19	0.63	0.72	0.59	0.85	0.84**	Linear
20:5 ω3	90.9	0.13	0.19	0.24	0.40	0.49	0.56	0.68	0.89**	Linear
	79.5	0.13	0.13	0.21	0.30	0.40	0.28	0.57	0.79**	Linear
	68.0	0.13	0.17	0.20	0.17	0.28	0.24	0.33	0.74**	Linear
20:4 ω3	90.9	0.08	0.14	0.15	0.28	0.33	0.27	0.38	0.76**	Linear
	79.5	0.08	0.10	0.13	0.20	0.23	0.23	0.37	0.84**	Linear
	68.0	0.08	0.10	0.12	0.26	0.20	0.22	0.19	0.41	•--
18:4 ω3	90.9	0.13	0.12	0.12	0.17	0.17	0.19	0.20	0.58**	Linear
	79.5	0.13	0.18	0.11	0.14	0.13	0.34	0.19	0.36	--
	68.0	0.13	0.07	0.11	0.09	0.15	0.13	0.10	0.18	--
18:3 ω3	90.9	1.12	1.24	1.23	1.26	1.32	1.29	1.51	0.49*	Linear
	79.5	1.12	1.11	1.31	1.33	1.32	1.29	1.34	0.72**	Linear
	68.0	1.12	1.24	1.25	1.20	1.12	1.18	1.24	0.08	--
22:5 ω6	90.9	0.02	trace quantities						--	--
	79.5								--	--
	68.0								--	--
22:4 ω6	90.9	0.11	0.08	0.05	0.11	0.11	0.04	0.08	--	--
	79.5	0.11	0.09	0.07	0.10	0.06	0.07	--	--	--
	68.0	0.11	0.05	0.13	0	0.11	0.11	0.11	--	--
20:4 ω6	90.9	0.49	0.44	0.36	0.44	0.43	0.35	0.43	−0.26	--
	79.5	0.49	0.43	0.44	0.42	0.41	0.38	0.44	−0.28	--
	68.0	0.49	0.42	0.53	0.19	0.45	0.51	0.34	−0.16	--
20:2 ω6	90.9	Not identified							--	--
	79.5								--	--
	68.0								--	--
18:2 ω6	90.9	18.99	19.80	19.58	19.33	19.90	19.20	20.72	0.04	--
	79.5	18.99	17.28	21.56	20.43	20.39	19.32	19.26	0.13	--
	68.0	18.99	20.31	21.27	19.35	18.60	19.89	18.22	−0.36	--

* $P < .05$ ** $P < .01$

these data indicate that, where a correlation existed, the data relative to the incorporation of the ω3 fatty acids were linear.

EFFECT OF MENHADEN OIL CONSUMPTION ON ORGANOLEPTIC EVALUATION OF PIG TISSUE

This second part of the study was made to determine (1) the organoleptic effect on the meat of the pigs fed different levels of menhaden oil in their diet and (2) the retention or disappearance of off-flavors by removal of menhaden oil from the diet of the pigs when they attained a body weight of 68.0, 79.5, or 90.9 kg.

TRIAL I: LEVEL OF MENHADEN OIL THAT RESULTS IN A FISHY FLAVOR

As was just indicated, Trial I was conducted to determine if menhaden oil fed at various levels in the diet would cause off- (fishy) flavor in pork. The levels used in the experiment bracketed a 1.0% level, which was reported by Vestal, Shrewsbury, Jordon, and Milligan (1945) to cause a fishy flavor.

Experimental Procedure

Reported here are the diets, management, samples, and tests.

TABLE 10.—*Longissimus dorsi* tissue: concentration of fatty acids found in *longissimus dorsi* tissue and correlation to quantity of various fatty acids fed for various time intervals.

Fatty acid	Pig weight group	Concentration of fatty acid in *longissimus dorsi* tissue when the percentage of menhaden oil in the diet was:							Correlation coefficient	Kind of regression line
		0	0.4	0.6	0.8	1.0	1.2	1.4		
	Kg	— — — — — — — — *Area percent of fatty acid* — — — — — — — —								
22:6 ω3	90.9	0.23	0.94	0.79	0.78	0.91	0.89	0.51	−0.09	--
	79.5	0.23	0.37	0.59	0.58	0.64	1.26	0.90	0.46	--
	68.0	0.23	0.47	0.47	0.25	0.42	0.48	0.85	0.46	--
22:5 ω3	90.9	0.32	1.15	1.34	0.85	1.02	0.85	0.85	--	--
	79.5	0.32	0.76	0.85	0.94	0.79	1.70	1.25	0.75**	Linear
	68.0	0.32	1.05	0.59	0.47	0.78	0.57	1.14	0.25	--
20:5 ω3	90.9	0.19	1.61	1.68	1.09	1.23	1.24	1.31	0.01	--
	79.5	0.19	0.47	0.98	0.58	0.52	1.88	1.47	0.43	--
	68.0	0.19	0.30	0.69	0.51	0.63	0.44	0.77	0.59*	Linear
20:4 ω3	90.9	0.53	2.42	2.87	0.92	0.91	0.89	0.87	−0.34	--
	79.5	0.53	0.23	1.24	2.30	0.21	1.51	0.28	0.05	--
	68.0	0.53	0.12	1.04	0.94	0.94	2.15	3.03	0.10	--
18:4 ω3	90.9	0.04	0.08	0.09	0.03	--	0.07	0.10	--	--
	79.5	0.04	0.05	--	0.24	--	--	--	--	--
	68.0	0.04	0.04	--	0.10	0.16	0.16	--	--	--
18:3 ω3	90.9	0.45	0.53	0.64	0.56	0.58	0.56	0.57	0.07	--
	79.5	0.45	0.38	0.53	0.62	0.43	0.63	0.44	0.20	--
	68.0	0.45	0.77	1.05	0.81	0.57	0.51	0.50	−0.23	--
22:5 ω6	90.9	0.02	0.06	0.09	0.12	--	--	--	−0.22	--
	79.5	0.02	tr	tr	0.18	tr	tr	tr	--	--
	68.0	0.02	tr	tr	0.04	0.29	tr	tr	--	--
22:4 ω6	90.9	0.34	0.41	0.84	0.37	--	0.32	0.16	−0.26	--
	79.5	0.34	0.48	0.40	0.22	0.15	0.19	0.16	0.09	--
	68.0	0.34	0.37	0.27	0.12	0.16	0.17	0.36	0.23	--
20:4 ω6	90.9	2.42	2.85	2.13	2.35	1.92	1.65	1.32	−0.58*	Linear
	79.5	2.42	2.04	3.00	1.62	1.52	3.55	2.64	0.10	--
	68.0	2.42	2.00	2.12	0.74	1.53	0.92	1.39	−0.44	--
20:2 ω6	90.9	0.13	0.19	0.25	0.09	tr	0.05	tr	−0.46*	Linear
	79.5	0.13	0.11	0.10	tr	0.05	tr	tr	−0.68**	Linear
	68.0	0.13	0.10	tr	0.05	0.11	tr	0.09	−0.21	--
18:2 ω6	90.9	9.46	12.54	10.18	10.96	11.08	10.34	9.65	−0.25	--
	79.5	9.46	10.56	13.39	9.45	11.44	17.35	13.84	0.53*	Linear
	68.0	9.46	15.63	10.14	12.36	10.39	8.81	9.61	−0.39	--

* $P < .05$ ** $P < .01$

Table 1 shows how the diets used were formulated, and Table 2 shows the gas-liquid-chromatographic analyses of the menhaden oil and the diets fed. Management and allotment are described in Table 11.

The right loin of each animal was collected, and three slices, each 1.37 cm thick, were cut proceeding posteriorly from the 10th rib. The slices and the remaining portion of the loin were held in frozen storage until used in the taste tests to be described shortly.

The slices of loin were placed in uncovered pans, one-half cup of water was added to each pan, and the pans were held for 50 min in a gas oven at 163.0° C. No seasoning was added to the samples. The remaining portions of the loins, which were used in a home-consumer test described in the next section, were prepared

TABLE 11.—Design of Trial I to determine the level of menhaden oil in the diet that will impart a fishy flavor to the meat of pigs.

Level of menhaden oil in the diet	Pigs allotted per treatment
%	*Number*
0	3
0.4	3
0.6	3
0.8	3
1.0	3
1.2	3
1.4	3

using variable cooking times and temperatures. Salt and pepper were the only condiments used.

Two tests were made: a panel test and a home-consumer test. Twelve panel members tested (once daily) a portion of the loin from various animals in a triangular test pattern. In addition to matching like samples, the panel members indicated a score according to the numerical standard: 1 (good) to 10 (inedible) and made any additional subjective comments that they felt would be helpful concerning the samples. The remaining portions of the loins were distributed randomly to staff members and were accompanied with a form requesting a description of the method of cooking used, a statement of the number of persons tasting, and a subjective evaluation of the flavor.

Results and Discussion

Tables 12, 13, and 14 present the results of Trial I, which was conducted to establish the level of fish oil in the diet that would induce a fishy flavor in pork. The results, as presented in Table 13, are somewhat misleading, because two facts need to be considered in interpreting them. First, part way through the taste test, the freezer in which the test samples were stored malfunctioned. The samples of meat thawed for 2 days and then refroze. Subsequently, a number of panelists detected off-flavors in the control sample as well as in the samples from the pigs receiving the lower levels of fish oil. Second, one panelist continuously detected off-flavor and fishiness regardless of the dietary treatment. In view of these two facts, Table 14 is included; here results are presented of tests conducted before the freezer malfunctioned and without the evaluations of the one panelist. The organoleptic results (Table 14) indicate that an off-flavor in pork could be detected when pigs consumed menhaden oil at a level of 0.8% of their diet and that a fishy flavor could be detected when the pigs consumed menhaden oil at a level of 1.2% of their diet and were fed when they attained a weight of 40.5 kg until they attained a market weight of 90.9 kg.

These results confirm a previous report by Vestal et al. (1945), which established that a level of 1.0% menhaden oil in the diet would cause a fishy flavor.

The results of the home-consumer test agree in general with those of the panel test that a fishy flavor was detected when menhaden oil was fed at a level of 1.2% in the diet. The one indication of off-flavor and fishy flavor in the control sample was found by the judge who had consistently done so in the panel test. All samples in the home-consumer test had been subjected to the thawing and refreezing process.

TABLE 12.—Panel test Trial I—organoleptic results obtained with loins of pigs fed menhaden oil at various levels in the diet.

Concentration of menhaden oil in diet	Samples tested	Detection of adverse flavor	
		Off	Fishy
%	*Number*	*Number*	*Number*
0	18	14	3
0.4	3	7	1
0.6	3	4	1
0.8	3	10	2
1.0	3	5	1
1.2	3	10	5
1.4	3	13	3

TABLE 13.—Panel test Trial I—organoleptic results (selected data) obtained with loins of pigs fed menhaden oil at various levels in the diet.

Concentration of menhaden oil in diet	Sample tested	Detection of adverse flavor	
		Off	Fishy
%	*Number*	*Number*	*Number*
0	11	0	0
0.4	1	0	0
0.6	2	2	0
0.8	2	2	0
1.0	3	4	0
1.2	2	4	2
1.4	1	6	1

TABLE 14.—Home-consumer test Trial I—organoleptic results obtained with loins of pigs fed menhaden oil at various levels in the diet.

Concentration of menhaden oil in diet	Samples tested	Testers	Detection of adverse flavor	
			Off	Fishy
%	*Number*	*Number*	*Number*	*Number*
0	14	48	1	1
0.4	6	28	6	0
0.6	6	12	0	0
0.8	6	18	1	0
1.0	6	25	1	0
1.2	5	9	2	2
1.4	3	22	7	7

The fact that persons unaware of the feeding regimen were less able to detect the off-flavor may indicate that the members of the test panel were overly critical in their evaluation. In view of the subjectivity of organoleptic tests, we felt that the aim of the trial was attained and that a suitable gradient in the concentration of fish oil in the diet was established for the further study of fishy flavor.

TRIAL II: RELATION OF FLAVOR OF MEAT TO BODY WEIGHT AT TIME MENHADEN OIL WAS REMOVED FROM DIET

As in Trial I, in Trial II loin samples were used in two organoleptic tests (panel and home-consumer) to determine if any fishy taste was imparted to animals fed the experimental diets. Twelve panel members tested six loin samples (*longissimus dorsi* and inner backfat) per day during the test. The panelists were asked to pick the control and to score each sample (lean and fat) on a numerical scale of 1 (good) to 5 (inedible). In addition, the members of the panel were asked to describe, subjectively, the flavor of the samples. All panelists were aware of the experimental design.

A home-consumer test was conducted in the same way as in Trial I.

The diets used were formulated and prepared in a manner similar to that indicated in Table 1. Table 2 shows the gas-liquid chromatographic analyses of the oil and of the diets fed.

The samples were collected and prepared as in Trial I.

The results of both organoleptic tests (panel and home-consumer, Tables 15 and 16) agree with those obtained in Trial I. In the panel tests, the results indicate that off-flavors were detected at the 0.8% level of menhaden oil in the diet and that a fishy flavor was detected at the 1.0% level.

In the home-consumer tests, a fishy flavor was not detected until the pigs were fed menhaden oil at the 1.2% level in the diet.

TABLE 15.—Panel test Trial II—organoleptic results obtained with *longissimus dorsi* and inner backfat tissue of pigs fed various levels of menhaden oil in the diet until the pigs attained a body weight of 90.9, 79.5, or 68.0 kg.

Concentration of menhaden oil in diet	Weight of pigs when oil was omitted from diet	Samples tested	Detection of adverse flavor	
			Off	Fishy
%	*Kg*	*Number*	*Number*	*Number*
0	--	7	0	[1]
0.4	90.9	3	1	1
	79.5	2	0	2
	68.0	2	0	0
0.6	90.9	3	0	0
	79.5	3	0	0
	68.0	1	0	0
0.8	90.9	3	0	0
	79.5	2	0	1
	68.0	2	1	2
1.0	90.0	3	0	0
	79.5	2	0	1
	68.0	2	3	6
1.2	90.9	4	0	0
	79.5	2	2	2
	68.0	1	3	3
1.4	90.9	2	0	0
	79.5	2	1	2
	68.0	2	0	0

[1] As was indicated in Trial I, one panelist detected off-flavor and fishy flavor in the control sample and in samples from pigs fed the low levels of menhaden oil in the diet. Because this panelist was unable to distinguish between the control and the test samples, he was replaced.

TABLE 16.—Home-consumer test Trial II—organoleptic results obtained with *longissimus dorsi* and inner backfat tissue of pigs fed various dietary levels of menhaden oil in the diet until the pigs attained a body weight of 90.9, 79.5, and 68.0 kg.

Concentration of menhaden oil in diet	Weight of pigs when oil was omitted from diet	Samples tested	Number of testers	Detection of adverse flavor	
				Off	Fishy
%	*Kg*	*Number*	*Number*	*Number*	*Number*
0	--	7	21	0	0
0.4	90.9	3	15	0	0
	79.5	2	3	0	0
	68.0	2	12	0	0
0.6	90.9	3	8	0	0
	79.5	3	11	0	0
	68.0	1	5	0	0
0.8	90.9	3	8	0	0
	79.5	2	6	0	0
	68.0	2	4	2	0
1.0	90.9	3	10	0	0
	79.5	2	9	5	0
	68.0	2	12	0	0
1.2	90.9	4	19	3	3
	79.5	2	5	0	0
	68.0	1	4	0	0
1.4	90.9	2	4	2	0
	79.5	2	3	3	3
	68.0	2	8	0	0

RELATION OF MENHADEN OIL FATTY ACIDS DEPOSITED TO ORGANOLEPTIC VALUES OBTAINED WITH PIG TISSUES

This third part of the study was made to determine if a relation exists between the degree of off-flavor detection and the fatty acid deposition pattern of the samples of pig tissue.

PROCEDURE

The details of management, patterns of fatty acid deposition in the tissues, and organoleptic tests were the same as those described earlier.

To establish the relation (if any) of the characteristic polyunsaturated ω3 fatty acids of menhaden oil to the off-flavor of pig tissue, we first had to establish a positive correlation (if any) between the concentrations of these fatty acids fed to the pigs to the concentrations of the fatty acids deposited in the various pig tissues. Once such a correlation (if it existed) was established, then the transformational relation of the concentration of fatty acids fed and deposited to the organoleptic evaluation could be undertaken.

Results of gas-liquid chromatographic analyses of the diets fed (Table 2) indicate that, in general, as the percentage of menhaden oil in the diet increased, the percentage of linolenic ω3 family acids (18:3 ω3, 18:4 ω3, 20:4 ω3, 22:5 ω3, and 22:6 ω3) characteristic of menhaden oil increased proportionately in the diet.

To determine whether the concentrations of these fatty acids in the diet are correlated with the taste of the pig flesh, we had to develop a weighted numerical score of organoleptic data. We obtained the weighted score for each sample tested by multiplying the number of testers times the numerical values of their scores and summing to a total. For example, if five of the panelists scored the sample 3 and if seven scored the sample 4, the weighted score would be $5 \times 3 = 15$ plus $7 \times 4 = 28$, or a total of $15 + 28 = 43$. The weighted scores were used as the Y axis, and the quantity of oil in kilograms or in percent consumed by each pig was used as the X axis in a subsequent correlation and polynomial regression analysis.

Although four tissues were examined with respect to the deposition of ω3 fatty acid, only two of these tissues (the inner backfat and the *longissimus dorsi*) were evaluated organoleptically. This comparison was further limited in view of the lack of correlation between the amount in the diet of ω3 fatty acids fed and the concentration of these fatty acids deposited in the *longissimus dorsi* (Tables 17, 18, and 19). Consequently, the relation of the concentration of the ω3 family fatty acids deposited in the inner backfat and the organoleptic score obtained with this tissue was used for the comparison of the relation of the concentration of the ω3 family fatty acids to the organoleptic score.

Because all six of the marine polyunsaturated (ω3) family fatty acids deposited in the inner backfat tissue were positively correlated with

TABLE 17.—Pigs fed to 90.9 kg [correlation and polynomial regression analyses of menhaden oil consumed (X) to individual fatty acids deposited (Y) in *longissimus dorsi* tissue of pigs when oil was fed until the pigs attained a body weight of 90.9 kg].

Fatty acid fed and deposited	Correlation coefficient	Regression coefficient	Standard error of regression	Last degree of polynomial significant	
				Degree	F value
22:6 ω3	−0.09	−0.016	0.041	--	0.15
22:5 ω3	--	--	--	--	--
22:5 ω6	−0.22	−0.038	0.041	--	0.85
22:4 ω6	−0.26	−0.052	0.048	--	1.19
20:5 ω3	0.01	0.003	0.077	--	0.00
20:4 ω3	−0.34	−0.029	0.191	--	2.26
22:1 (?)	--	--	--	--	--
20:4 ω6	−0.58**	−0.220	0.074	Linear	8.76**
20:3	−0.22	−0.022	0.024	--	0.84
21:1 ω9	−0.32	−0.029	0.021	--	1.96
20:2 ω6	−0.46*	−0.036	0.017	Linear	4.56*
20:2 ω9	−0.34	−0.072	0.048	--	2.24
18:4 ω3	--	--	--	--	--
20:1 ω9	0.02	0.002	0.024	--	0.01
18:3 ω3	0.07	0.004	0.014	--	0.09
20:0	−0.01	−0.001	0.016	--	0.00
18:2 ω6	−0.25	−0.210	0.194	--	1.15
19:0	0.29	0.047	0.039	--	1.50
18:1 ω9	0.46*	1.130	0.527	Linear	4.59*
18:0	0.09	--	--	--	--
16:2	−0.04	−0.002	0.013	--	0.02
17:0	−0.05	−0.009	0.045	--	0.04
16:1 ω7	−0.09	−0.035	0.099	--	0.13
16:0	−0.04	−0.054	0.300	--	0.03
15:1	--	--	--	--	--
15:0	−0.22	−0.026	0.027	--	0.89
14:1	−0.06	−0.009	0.038	--	0.06
14:0	−0.20	−0.033	0.038	--	0.74

* $P < .05$ ** $P < .01$

the concentration of menhaden oil in the diet fed, the quantity of oil consumed (X) could be compared with the organoleptic scores (Y) obtained for the pigs in each weight group (68.0, 79.5, 90.9 kg).

RESULTS

Tables 20, 21, and 22 give the weighted organoleptic scores obtained from the panel organoleptic tests. (Larger numerical values indicate an unacceptable product or a trend toward an unacceptable product.)

Statistical analyses of these data indicate a positive correlation between increased consumption of oil and higher organoleptic scores for tissues from pigs fed the oil until they attained a body weight of 90.9 kg (Table 23). Removal of the oil from the diet of the pigs at a body weight of either 68.0 or 79.5 kg resulted in a

TABLE 18.—Pigs fed to a body weight of 79.5 kg [correlation and polynomial regression analyses of menhaden oil consumed (X) to individual fatty acids deposited (Y) in *longissimus dorsi* tissue of pigs when oil was fed until the pigs attained a body weight of 79.5 kg].

Fatty acid fed and deposited	Correlation coefficient	Regression coefficient	Standard error of regression	Last degree of polynomial significant	
				Degree	F value
22:6 ω3	0.46	0.189	0.104	--	3.29
22:5 ω3	0.75**	0.235	0.059	Linear	15.82**
22:5 ω6	--	--	--	--	--
22:4 ω6	0.09	0.012	0.039	--	0.10
20:5 ω3	0.43	0.170	0.103	--	2.73
20:4 ω3	0.05	0.036	0.224	--	0.03
22:1 (?)	--	--	--	--	--
20:4 ω6	0.10	0.069	0.209	--	0.11
20:3	0.27	0.028	0.029	--	0.91
21:1 ω9	−0.65**	−0.039	0.013	Linear	8.70*
20:2 ω6	−0.68**	−0.039	0.012	Linear	10.47**
20:2 ω9	−0.39	−0.123	0.084	--	2.17
18:4 ω3	--	--	--	--	--
20:1 ω9	−0.45	−0.092	0.053	--	3.05
18:3 ω3	0.20	0.020	0.027	--	0.51
20:0	−0.05	−0.006	0.029	--	0.04
18:2 ω6	0.53*	1.216	0.557	Linear	4.77*
19:0	−0.02	−0.002	0.027	--	0.00
18:1 ω9	−0.36	−1.085	0.815	--	1.77
18:0	−0.17	−0.172	0.290	--	0.35
16:2	0.11	0.014	0.037	--	0.14
17:0	−0.11	−0.017	0.043	--	0.15
16:1 ω7	−0.32	−0.101	0.088	--	1.33
16:0	−0.58*	−0.918	0.372	Linear	6.09*
15:1	--	--	--	--	--
15:0	−0.04	−0.006	0.046	--	0.02
14:1	0.32	0.038	0.032	--	1.35
14:0	−0.26	−0.039	0.043	--	0.84

* $P < .05$ ** $P < .01$

TABLE 19.—Pigs fed to a body weight of 68.0 kg [correlation and polynomial regression on analyses of menhaden oil consumed (X) to individual fatty acids deposited (Y) in *longissimus dorsi* tissue of pigs when oil was fed until the pigs attained a body weight of 68.0 kg].

Fatty acid fed and deposited	Correlation coefficient	Regression coefficient	Standard error of regression	Last degree of polynomial significant	
				Degree	F value
22:6 ω3	0.46	0.113	0.073	--	2.38
22:5 ω3	0.24	0.087	0.120	--	0.53
22:5 ω6	--	--	--	--	--
22:4 ω6	0.23	0.049	0.068	--	0.52
20:5 ω3	0.59*	0.130	0.059	--	4.79
20:4 ω3	0.10	0.201	0.680	--	0.09
22:1 (?)	--	--	--	--	--
20:4 ω6	−0.44	−0.363	0.247	--	2.16
20:3	−0.20	−0.029	0.047	--	0.38
21:1 ω9	0.38	0.039	0.032	--	1.50
20:2 ω6	−0.21	−0.012	0.019	--	0.41
20:2 ω9	−0.43	−0.301	0.212	--	2.02
18:4 ω3	--	--	--	--	--
20:1 ω9	−0.42	−0.139	0.101	--	1.91
18:3 ω3	−0.23	−0.056	0.078	--	0.51
20:0	−0.27	−0.042	0.050	--	0.68
18:2 ω6	−0.39	−1.081	0.845	--	1.64
19:0	−0.24	−0.057	0.077	--	0.55
18:1 ω9	0.44	1.392	0.944	--	2.17
18:0	0.40	0.556	0.422	--	1.73
16:2	−0.13	−0.036	0.094	--	0.14
17:0	−0.12	−0.029	0.077	--	0.14
16:1 ω7	0.08	0.080	0.313	--	0.06
16:0	−0.16	−0.531	1.065	--	0.25
15:1	--	--	--	--	--
15:0	−0.03	−0.013	0.129	--	0.01
14:1	−0.08	−0.019	0.080	--	0.06
14:0	−0.11	−0.029	0.083	--	0.12

* $P < .05$ ** $P < .01$

TABLE 20.—Panel test Trial II—weighted organoleptic scores obtained with inner backfat of pigs fed various levels of menhaden oil in the diet until the pigs attained a body weight of 90.9 kg.

Quantity of oil consumed (X)		Weighted organoleptic score (Y)
Kg	*As % of diet*	
0	0	21
0.81		23
.83	0.4	21
.91		27
1.26		22
1.29	0.6	22
1.36		25
1.77		31
1.86	0.8	26
2.07		32
2.00		30
2.15	1.0	34
2.35		35
2.55		29
2.74	1.2	36
2.74		28
2.82		--
3.20	1.4	37
3.25		32

TABLE 21.—Panel test Trial II—weighted organoleptic scores obtained with inner backfat of pigs fed various levels of menhaden oil in the diet until the pigs attained a body weight of 79.5 kg.

Quantity of oil consumed (*X*)		Weighted organoleptic score (*Y*)
Kg	*As % of diet*	
0	0	21
0.55	0.4	25
.64		27
.94	0.6	20
.99		21
.99		18
1.30	0.8	20
1.34		20
1.36	1.0	35
1.48		23
1.77	1.2	34
2.11	1.4	32

TABLE 22.—Panel test Trial II—weighted organoleptic scores obtained with inner backfat of pigs fed various levels of menhaden oil in the diet until the pigs attained a body weight of 68.0 kg.

Quantity of oil consumed (*X*)		Weighted organoleptic score (*Y*)
Kg	*As % of diet*	
0	0	21
0.45		23
.54	0.4	20
.72	0.6	21
.98	0.8	23
1.09		22
1.21	1.0	22
1.21		25
1.54	1.2	26
1.61	1.4	23
1.61		17

TABLE 23.—Correlation and polynomial regression analyses of quantity of menhaden oil consumed (*X*) to weighted organoleptic score (*Y*) when the oil was fed until the pigs attained a body weight of 90.9, 79.5, or 68.0 kg.

Oil fed to	Correlation coefficient	Regression coefficient	Standard error of regression	Last degree of polynomial significant	
				Degree	*F* value
Kg					
90.9	0.82**	2.169	0.371	Linear	329.58**
79.5	.49	2.375	1.325	--	3.21
68.0	.21	0.617	0.967	--	0.41

** $P < .01$

loss of the significant positive correlation between the variables, although the correlation coefficient obtained for the group weighing 79.5 kg approached significance.

These results of organoleptic tests are in agreement with reports of Miller et al. (1967), which indicate that ω3 family fatty acicds, when fed and subsequently deposited, are positively correlated with organoleptic scores obtained with broiler flesh. The results are in partial agreement with the hypothesis of Banks and Hilditch (1932), who suggested that the fatty acids of the C_{20-22} series are associated with an off- (fishy) flavor. Both the results reported here and those reported by Miller et al. (1967) indicate that fatty acids of the ω3 family containing 18 to 22 carbon atoms are positively correlated with the incidence and degree of off-flavor in pig or broiler flesh. These fatty acids may be causal agents for the off-flavor, or they may not be. In fact, they probably are the precursors of the compound producing the off-flavor.

In these experiments, the inclusion of the menhaden oil in the diet of the pigs resulted in no physiological abnormalities other than the production of off-flavor and an alteration in the pattern of fatty acids in the tissues. This result was not unexpected, because previous work at the National Marine Fisheries Service Technological Laboratory at College Park had indicated that levels of menhaden oil in excess of 10% of the diet are necessary to produce the physiological abnormalities of exudative diathesis and muscular dystrophy experimentally. Adding various antioxidants (vitamin E, selenium, and ethoxyquin) to the diet at compensatory levels prevented the development of these abnormalities (exudative diathesis and muscular dystrophy) in chicks fed menhaden oil at high concentrations (Miller, Leong, Knobl, and Gruger, 1965).

METABOLIC INTERACTIONS OF FATTY ACIDS OF THE OMEGA FAMILY (ω3, ω6, ω9)

Mohrhauer and Holman (1963a), Rahm and Holman (1964), Tinsley (1964), and Lowry and Tinsley (1966) have demonstrated that feeding

rats increasingly higher concentrations of linolenic acid (18:3 ω3) increases the concentration of the fatty acids of the ω3 family in the liver and that the proportion of the fatty acids of the oleic (18:1 ω9) and linoleic (18:2 ω6) families are concomitantly reduced. They hypothesize that this interaction is due to the competition for enzymes necessary for elongation and desaturation within the individual families of fatty acids.

Since our pig experiment included an increasing quantity of 18:3 ω3 in the diet, the question arose as to whether this hypothesized competitive interaction actually occurred.

Trial II results were analyzed by correlation analysis and polynomial regression analysis as previously described. The quantity of menhaden oil consumed constituted the X axis, and the quantity of the 17:1 ω9 or 18:2 ω6 family fatty acid in question the Y axis.

The ω3 family fatty acids incorporated into the diet of the pigs as menhaden oil and subsequently ingested resulted in a significantly depressed deposition of the quantity of certain members of the ω6 and ω9 families of fatty acids. The mechanism involved, according to the accepted hypothesis, is that the parent fatty acids of the various fatty acid families trigger a highly competitive mechanism for the metabolic enzymes of the systems of carbon-chain elongation and dehydrogenation. Successful competition for the enzymes depends upon an affinity preference (ω3, ω6, and ω9) and upon the relative concentration of the various fatty acids, or upon both affinity and concentration. These results agree in part with the experimental evidence (Mohrhauer and Holman, 1963a) whereby the feeding of increasing levels of one of the parent acids or other members of a family results in an accumulation of acids

TABLE 24.—Liver tissue—comparison of correlation coefficients and significant degree of polynomial regression obtained by relating the quantity of menhaden oil consumed (X) until the pigs attained body weights of 90.9, 79.5, or 68.0 kg to the amount of individual fatty acids deposited in liver tissue (Y).

Fatty acid fed and deposited	Correlation coefficients when oil is fed until the pigs weighed:			Last degree of polynomial significant when oil was fed until the pigs weighed:					
				90.9 kg		79.5 kg		68.0 kg	
	90.9 kg	79.5 kg	68.0 kg	Degree	F value	Degree	F value	Degree	F value
22:6 ω3	0.69**	0.70**	0.65*	Quadratic	7.78*	Linear	11.52**	Linear	6.46*
22:5 ω3	0.78**	0.56*	0.59*	Quadratic	5.94*	Linear	5.49*	--	4.68
22:5ω6	−0.27	−0.60*	−0.83**	--	0.92	Linear	6.73*	Linear	19.30**
22:4 ω6	−0.65**	−0.63*	−0.69*	Cubic	58.73**	Cubic	5.38*	Linear	8.08**
20:5 ω3	0.89**	0.50	0.67*	Quadratic	14.53**	--	3.98	Linear	7.45**
20:4 ω3	−0.21	−0.42	0.39	--	0.77	--	2.57	--	1.63
22:1 (?)	−0.40	−0.23	0.23	--	3.30	--	0.68	--	0.49
20:4 ω6	−0.70**	−0.19	−0.74**	Quadratic	6.62*	--	0.46	Linear	10.79**
20:3	−0.14	0.36	0.43	--	0.32	--	1.74	--	2.02
21:1 ω9	−0.59**	−0.40	−0.47	Linear	9.19**	--	2.26	--	2.56
20:2 ω6	−0.52*	−0.34	0.13	Cubic	7.59*	--	1.60	--	0.15
20:2 ω9	−0.12	−0.48	0.52	--	0.26	--	3.61	--	3.42
18:4 ω3	−0.00	−0.29	0.33	--	0.00	--	1.11	--	1.06
20:1 ω9	−0.22	−0.38	−0.28	--	0.87	--	2.04	--	0.77
18:3 ω3	0.26	−0.39	0.12	--	1.20	--	2.14	--	0.13
20:0	0.16	−0.22	0.07	--	0.47	--	0.61	--	0.05
18:2 ω6	−0.33	0.16	−0.12	--	2.10	--	0.31	--	0.13
19:0	0.10	0.03	0.43	--	0.16	--	0.01	--	2.02
18:1 ω9	−0.09	−0.49	−0.54	--	0.15	--	3.80	--	3.76
18:0	−0.06	0.33	−0.07	--	0.06	--	1.46	--	0.05
16:2	0.24	−0.30	0.47	--	1.08	--	1.20	--	2.53
17:0	0.13	0.02	0.47	--	0.31	--	0.00	--	2.48
16:1 ω7	0.02	−0.49	−0.12	--	0.01	--	3.72	--	0.13
16:0	−0.26	−0.42	−0.22	--	1.25	--	2.56	--	0.46
15:1	0.10	−0.13	0.45	--	0.17	--	0.22	--	2.25
15:0	−0.28	−0.27	0.75**	--	1.44	--	0.94	Linear	11.76**
14:1	−0.01	−0.27	0.55	--	0.00	--	0.96	--	3.84
14:0	0.01	−0.47	0.06	--	0.00	--	3.32	--	0.03

* $P < .05$ ** $P < .01$

TABLE 25.—Inner backfat tissue—comparison of correlation coefficients and significant degree of polynomial regression obtained by relating the quantity of menhaden oil consumed (X) until the pigs attained a body weight of 90.9, 79.5, or 68.0 kg to the amount of individual fatty acids deposited in inner backfat tissue (Y).

Fatty acid fed and deposited	Correlation coefficients when oil is fed until the pigs weighed:			Last degree of polynomial significant when oil was fed until the pigs weighed:					
				90.9 kg		79.5 kg		68.0 kg	
	90.9 kg	79.5 kg	68.0 kg	Degree	F value	Degree	F value	Degree	F value
22:6 ω3	0.76**	0.58*	0.49	Linear	23.54**	Linear	6.02*	--	2.87
22:5 ω3	0.77**	−0.01	0.71**	Linear	24.94**	--	0.00	Linear	9.28*
22:5ω6	--	--	--	--	--	--	--	--	--
22:4 ω6	--	--	--	--	--	--	--	--	--
20:5 ω3	0.71**	0.55*	0.71**	Linear	17.64**	Linear	5.31*	Linear	9.33*
20:4 ω3	0.72**	0.43	0.89**	Linear	18.15**	--	2.70	Linear	35.51**
22:1 (?)	--	--	--	--	--	--	--	--	--
20:4 ω6	0.03	0.09	−0.60*	--	0.02	--	0.10	Linear	4.99*
20:3	−0.02	0.11	0.13	--	0.01	--	0.15	--	0.16
21:1 ω9	--	--	--	--	--	--	--	--	--
20:2 ω6	--	--	--	--	--	--	--	--	--
20:2 ω9	−0.28	−0.25	−0.32	--	1.43	--	0.77	--	1.06
18:4 ω3	0.70**	0.15	0.54	Linear	16.65**	--	0.26	--	3.77
20:1 ω9	−0.48*	−0.31	−0.32	Linear	5.19*	--	1.25	--	0.99
18:3 ω3	0.52*	0.17	0.52	Linear	6.46*	--	0.36	--	3.30
20:0	−0.33	0.01	−0.07	--	2.01	--	0.00	--	0.05
18:2 ω6	0.41	0.10	−0.01	--	3.49	--	0.11	--	0.00
19:0	--	--	--	--	--	--	--	--	--
18:1 ω9	−0.09	−0.37	−0.46	--	0.14	--	1.96	--	2.37
18:0	−0.21	−0.13	0.17	--	0.82	--	0.20	--	0.25
16:2	0.50*	−0.03	0.50	Linear	5.96*	--	0.01	--	2.96
17:0	0.49*	0.43	0.51	Linear	5.52*	--	2.72	--	3.22
16:1 ω7	0.31	−0.17	0.08	--	1.87	--	0.37	--	0.07
16:0	−0.24	0.37	0.21	--	1.06	--	1.94	--	0.40
15:1	−0.31	0.12	0.33	--	1.77	--	0.18	--	1.13
15:0	0.13	−0.25	0.19	--	0.28	--	0.79	--	0.34
14:1	−0.18	0.27	0.35	--	0.60	--	0.91	--	1.23
14:0	−0.24	0.19	0.46	--	1.03	--	0.43	--	2.39

* $P < .05$ ** $P < .01$

derived from these acids and in a concomitant decrease in the quantity of acids of the other families.

In Trial II, we demonstrated the interrelation of fatty acid families.

Specific evidence that the deposition of the ω6 and ω9 families are inhibited appears in Tables 24 to 27. As the quantity of the ω3 fatty acids fed and deposited increased, the quantity of the 20:2 ω6, 21:1 ω9, 20:4 ω6, and 22:4 ω6 fatty acids found in the various tissues decreased significantly. The linolenic (ω3) family fatty acids of the menhaden oil therefore inhibited the conversion of oleic (18:1 ω9) and linoleic (18:2 ω6) to the elongated and dehydrogenated members of their respective families by preventing the addition of two carbons or the removal of two hydrogens, or by preventing both the addition of two carbons and the removal of two hydrogens.

SUMMARY AND CONCLUSIONS

Pigs were fed diets containing fish oil in two feeding trials to investigate (1) the organoleptic effect produced in pig tissue by feeding pigs stabilized crude menhaden oil; (2) the possible retention or disappearance of off-flavor by withdrawing the fish oil from the diet at given times; (3) the nature of the fatty acid composition of the inner backfat tissues, the outer backfat tissues, the liver tissues, and the *longissimus dorsi* tissues; (4) the relation of composition to off-flavor (if an off-flavor is produced); and (5) the hypothesized metabolic interactions of fatty acid families.

An off-flavor and a fishy flavor were detected in the meat of pigs fed diets containing about 1% of menhaden oil.

Gas-liquid chromatographic analyses of the tissues indicated that up to 28 saturated and

TABLE 26.—Outer backfat tissue—comparison of correlation coefficients and significant degree of polynomial regression obtained by relating the quantity of menhaden oil consumed (X) until the pigs attained body weights of 90.9, 79.5, or 58.0 kg to the amount of individual fatty acids deposited in outer backfat tissue (Y).

Fatty acid fed and deposited	Correlation coefficients when oil is fed until the pigs weighed:			Last degree of polynomial significant when oil was fed until the pigs weighed:					
				90.9 kg		79.5 kg		68.0 kg	
	90.9 kg	79.5 kg	68.0 kg	Degree	F value	Degree	F value	Degree	F value
22:6 ω3	0.85**	0.58*	0.74**	Linear	43.30**	Linear	6.66*	Linear	11.15**
22:5 ω3	0.89**	0.85**	0.84**	Linear	66.64**	Linear	30.88**	Linear	21.23**
22:5 ω6	--	--	--	--	--	--	--	--	--
22:4 ω6	--	--	--	--	--	--	--	--	--
20:5 ω3	0.89**	0.79**	0.74**	Linear	63.25**	Linear	19.53**	Linear	11.12**
20:4 ω3	0.76**	0.84**	0.41	Linear	23.48**	Linear	28.60**	--	1.82
22:1 (?)	--	--	--	--	--	--	--	--	--
20:4 ω6	−0.26	−0.28	−0.16	--	1.25	--	0.98	--	0.24
20:3	−0.03	−0.12	−0.15	--	0.01	--	0.17	--	0.19
21:1 ω9	--	--	--	--	--	--	--	--	--
20:2 ω6	--	--	--	--	--	--	--	--	--
20:2 ω9	−0.36	−0.44	−0.37	--	2.57	--	2.84	--	1.43
18:4 ω3	0.58**	0.36	0.18	Linear	8.63**	--	1.75	--	0.32
20:1 ω9	−0.54**	−0.32	−0.37	Linear	7.14*	--	1.37	--	1.40
18:3 ω3	0.49**	0.72**	0.08	Linear	5.45*	Linear	13.09**	--	0.06
20:0	0.57**	0.73**	0.35	Linear	8.32**	Linear	13.62**	--	1.29
18:2 ω6	0.04	0.13	−0.36	--	0.03	--	0.22	--	1.35
19:0	--	--	--	--	--	--	--	--	--
18:1 ω9	−0.24	−0.47	−0.22	--	1.04	--	3.42	--	0.46
18:0	−0.13	−0.03	0.47	--	0.27	--	0.01	--	2.56
16:2	0.44*	−0.01	0.07	--	4.18	--	0.00	--	0.05
17:0	0.50*	0.06	0.24	Linear	5.66*	--	0.04	--	0.57
16:1 ω7	0.14	0.48	−0.40	--	0.33	--	3.59	--	1.70
16:0	−0.39	0.14	0.10	--	3.00	--	0.23	--	0.09
15:1	0.26	−0.18	0.06	--	1.28	--	0.40	--	0.40
15:0	0.43	0.04	0.08	--	3.89	--	0.02	--	0.06
14:1	0.32	−0.05	0.01	--	1.96	--	0.03	--	0.00
14:0	0.18	−0.25	−0.78**	--	0.56	--	0.81	Linear	14.14*

* $P < .05$ ** $P < .01$

unsaturated fatty acids were present. Unsaturated fatty acids from four of the fatty acid families (ω3, ω6, ω9, and ω7) were found in each of the tissues.

The quantity of the characteristic fatty acids (ω3) of the fish oil fed correlated significantly and positively with the quantity of these fatty acids deposited in the inner backfat tissues and the outer ones and the liver tissues, but not in the *longissimus dorsi* tissues.

Similarly, the quantity of these ω3 fatty acids fed correlated significantly and positively with a weighted organoleptic score of the inner backfat tissues. Removal of these fatty acids from the diet of the pigs at two different body weights —namely, 68.0 and 79.5 kg—prior to their being marketed at 90.9 kg resulted in a loss of significance by the correlation coefficients, although the correlation coefficients obtained were positive.

On a practical feeding basis, fish oil with similar fatty acid composition consumption should be limited to 0.8% of the diet if fed until pigs are marketed. If the oil is withdrawn from the diet prior to marketing, higher levels can be fed.

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TABLE 27.—*Longissimus dorsi* tissue—comparison of correlation coefficients and significant degree of polynomial regression obtained by relating the quantity of menhaden oil consumed (X) until the pigs attained body weights of 90.9, 79.5, or 68.0 kg to the amount of individual fatty acids deposited in *longissimus dorsi* tissue (Y).

Fatty acid fed and deposited	Correlation coefficients when oil is fed until the pigs weighed:			Last degree of polynomial significant when oil was fed until the pigs weighed:					
				90.9 kg		79.5 kg		68.0 kg	
	90.9 kg	79.5 kg	68.0 kg	Degree	F value	Degree	F value	Degree	F value
22:6 ω3	−0.09	0.46	0.46	--	0.15	--	3.29	--	2.38
22:5 ω3	−0.22	0.75**	0.24	--	--	Linear	15.82**	--	0.53
22:5 ω6	--	--	--	--	0.85	--	--	--	--
22:4 ω6	−0.26	0.09	0.23	--	1.19	--	0.10	--	0.52
20:5 ω3	0.01	0.43	0.59*	--	0.00	--	2.73	--	4.79
20:4 ω3	−0.34	0.05	0.10	--	2.26	--	0.03	--	0.09
22:1 (?)	--	--	--	--	--	--	--	--	--
20:4 ω6	−0.58**	0.10	−0.44	Linear	8.76**	--	0.11	--	2.16
20:3	−0.22	0.27	−0.20	--	0.84	--	0.91	--	0.38
21:1 ω9	−0.32	−0.65**	0.38	--	1.96	Linear	8.70*	--	1.50
20:2 ω6	−0.46*	−0.68**	−0.21	Linear	4.56*	Linear	10.47**	--	0.41
20:2 ω9	−0.34	−0.39	−0.43	--	2.24	--	2.17	--	2.02
18:4 ω3	--	--	--	--	--	--	--	--	--
20:1 ω9	0.02	−0.45	−0.42	--	0.01	--	3.05	--	1.91
18:3 ω3	0.07	0.20	−0.23	--	0.09	--	0.51	--	0.51
20:0	−0.01	−0.05	−0.27	--	0.00	--	0.04	--	0.68
18:2 ω6	−0.25	0.53*	−0.39	--	1.15	Linear	4.77*	--	1.64
19:0	0.29	−0.02	−0.24	--	1.50	--	0.00	--	0.55
18:1 ω9	0.46*	−0.36	0.44	Linear	4.59*	--	1.77	--	2.17
18:0	0.09	−0.17	0.40	--	--	--	0.35	--	1.73
16:2	−0.04	0.11	−0.13	--	0.02	--	0.14	--	0.14
17:0	−0.05	−0.11	−0.12	--	0.04	--	0.15	--	0.14
16:1 ω7	−0.09	−0.32	0.08	--	0.13	--	1.33	--	0.06
16:0	−0.04	−0.58*	−0.16	--	0.03	Linear	6.09*	--	0.25
15:1	--	--	--	--	--	--	--	--	--
15:0	−0.22	−0.04	−0.03	--	0.89	--	0.02	--	0.01
14:1	−0.06	0.32	−0.08	--	0.06	--	1.35	--	0.06
14:0	−0.20	−0.26	−0.11	--	0.74	--	0.84	--	0.12

* $P < .05$ ** $P < .01$

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CETACEANS FROM THE LESSER ANTILLEAN ISLAND OF ST. VINCENT

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ABSTRACT

A preliminary list of cetaceans collected and observed during the course of a fishery for blackfish or pilot whales (*Globicephala*) in the waters of the Lesser Antillean island of St. Vincent is presented and includes: *Megaptera novaeangliae, Steno bredanensis, Tursiops truncatus, Grampus griseus,* two species of *Stenella* to which specific names are not formally applied, *Feresa attenuata, Pseudorca crassidens, Globicephala macrorhyncha, Orcinus orca, Physeter catodon,* and *Ziphius cavirostris.* Nearest published records in the western Atlantic are given, as well as limited biological notes on some of the species. The taxonomic relationships of the two forms of *Stenella* are suggested and both species are illustrated. Landings of pilot whales in the fishery over a period of 9 years are included.

There is a small but active fishery for blackfish or pilot whales (*Globicephala*) centered around the village of Barrouallie on the western or lee side of the Lesser Antillean island of St. Vincent. While the major direction of this fishery is the pursuit of blackfish, intensive studies made by the writers independently and cooperatively over the past several years have shown that a number of other small cetaceans are captured as well. The primary purpose of the fishery is the production of meat and cooking oil, both used locally, and the species taken is not especially important to the fishermen, except that the larger animals are the more profitable. Hence the concentration on blackfish. Anything that is seen is pursued except the larger and fast baleen whales. The techniques and history of the blackfish fishery have been discussed by Brown (1945, 1947), Hickling (1950), Morice (1958), Allen (1966),[5] Morris (1966),[6] Jackson (1967),[7] Rathjen and Sullivan (1970), Caldwell and Caldwell (in press), and others. In brief it is conducted from small open boats launched daily from shore and powered primarily by sail and oar (see Rathjen and Sullivan, 1970). One motor launch recently has been employed and it produces the majority of the catches of the faster swimming small dolphins. Other motor launches, both inboard and outboard, are planned (Caldwell and Caldwell, in press). The cetaceans are taken both by hand harpoon and by small gun harpoons fired from a fixed stand on the bow of the boat.

We present here a summary of our findings to date regarding species taken in the fishery. The records of odontocetes are supported by skeletal remains and/or recognizable photographs of carcasses or parts of carcasses. Copies of all of the photographs mentioned below are in the Caldwells' files with duplicates of some in those of Rathjen and Sullivan. The skeletal material, unless otherwise stated, presently is being studied by the Caldwells at the Florida State Museum, Gainesville. The "SV" numbers associated with records discussed in the text are field numbers in the Caldwells' files. With more collecting and analysis of the results,

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[6] Morris, E. L. 1966. A brief history of Barrouallie from 1719 to present day (1966). St. Vincent Teachers College, Kingstown, St. Vincent, Unpublished report (file no. 39), 51 p.

[7] Jackson, L. R. 1967. The blackfish industry of Barrouallie, St. Vincent. St. Vincent Teachers College, Kingstown, St. Vincent, Unpublished report (file no. 26), 38 p.

Manuscript received January 1971.

both of which are ongoing, it should be possible to say more about some of the St. Vincent faunal elements than is appropriate now. Our purpose here is only to provide an annotated summary of the existing records for this island in order to give for the first time a relatively complete list of such a fauna from one specific locality in the Antilles and to provide a firmer basis for zoogeographic statements required for ongoing studies in this and other disciplines in the West Indies and Caribbean.

Although there are a number of individual records of cetaceans from the West Indies (in part summarized by Hershkovitz, 1966), or reports which include as many as three or four species, there are few reports which include enough of the expected species to give sufficient data for evaluating the local cetacean fauna. The best of these are from the northern Antilles (from Cuba by Cuni, 1918, and Aguayo, 1954; and from Puerto Rico to Antigua by Erdman, 1970).

Studies similar to ours on small cetaceans, but more detailed, have been conducted on the Atlantic coast of Africa in the vicinity of Senegal and to some lesser degree the Ivory Coast and the Cape Verde Islands. The odontocete cetacean fauna in those similar latitudes is remarkably similar to that of St. Vincent even though the two areas lie some 5000 km apart across the open sea. This similarity extends even to forms such as *Steno* and *Feresa* that are generally rare in collections. Cadenat and others have published a series of reports on their studies of the African fauna, but a list of the species found can be had by consulting a combination of two of these (Cadenat, 1949, 1959). The latter also summarizes much of the other literature on the cetaceans of the area. Van Bree and Cadenat (1968) in addition recorded *Peponocephala electra* from Senegal.

SPECIES ACCOUNTS

With the exception of a spotted dolphin, we follow Rice and Scheffer (1968) in our arrangement of species and in the scientific and/or common names applied to them.

Megaptera novaeangliae (BOROWSKI)— HUMPBACK WHALE

In early May 1968, an individual of adult size slowly passed close along the lee shore of St. Vincent in a southward direction. Several of the St. Vincent whalemen, familiar with this species from seeing it in a nearby humpback fishery at Bequia, observed this individual from boats but made no effort to harpoon it. The St. Vincent whalemen tell us that they see a few individuals of this species each year but that they never try to harpoon one because of the large size of these whales.

A few humpbacks are usually taken each year (mostly from February to April) in the Bequia fishery just to the south in the St. Vincent Grenadines which utilizes bomb guns in addition to hand harpoons. Accounts of the latter fishery were given by Brown (1945), Fenger (1958), Mitchell (1965), and Quashie (1966).[8] An early account of New England whaling vessels hunting humpbacks in the region was included by Lindeman (1880), but Clark (1887: pl. 183) for as early as 1880 included waters near St. Vincent on a map showing abandoned humpback whaling grounds. These and other reports mention *Megaptera* in St. Vincent and/or nearby waters.

Steno bredanensis (LESSON)— ROUGH-TOOTHED DOLPHIN

A skull (SV-1-SB) of a specimen of unknown size and sex was obtained from the fishery in the spring of 1969. This is the first record for this species from St. Vincent and from the Caribbean. We find no prior and contemporary basis for Kellogg's (1940: 69) inclusion of this species in the Caribbean fauna, nor for the indication by Hall and Kelson (1959: 819) that its range is continuous in the western Atlantic from Virginia to South America.

The records closest to St. Vincent in the western Atlantic are from off Havana, Cuba (and thus non-Caribbean) to the north, as *S. rostratus* (see Aguayo, 1954), and from an unstated locality off the Brazilian coast to the south (Ham-

[8] Quashie, I. N. 1966. The whale industry in Bequia. St. Vincent Teachers College, Kingstown, St. Vincent, Unpublished report (file no. 30), 32 p.

ilton, 1945). *S. fuscus* Gray, from an unstated area in Cuban waters, may be this species (True, 1889: 27) and if so would represent another Cuban record. However, the unique type of this latter species, a preserved fetus, apparently was lost even in True's time and the record cannot be substantiated. Presumably the previous Caribbean records of this species (see above) have been based on this Cuban material.

Tursiops truncatus (MONTAGU)—BOTTLENOSED DOLPHIN

The Caldwells have color photographs of the head of an immature specimen of unknown size and sex taken in the fishery on 17 May 1968. The head was obtained from a market where it had been split longitudinally in order to get to the brains, which are eaten. The skull (SV-1-TT) is also split but complete.

This species has not been reported from St. Vincent before, but Turner (1912: 135) listed the mandible (as *Tursiops tursio*) of a specimen from nearby Barbados.

Grampus griseus (G. CUVIER)—RISSO'S DOLPHIN OR GRAY GRAMPUS

The Caldwells have the skull (SV-1-GG) and color photographs of the intact head of a specimen of unknown size and sex taken in the fishery in the summer of 1968. They also have the skulls of two more specimens (SV-2-GG, SV-3-GG), of unknown size and sex, taken during the summer of 1970.

In addition, the Caldwells have black and white photographs (SV-4-GG) of an individual (also of unknown size and sex) taken in September or October 1967.

Until recently this species was considered a northern form in the western Atlantic, but specimens are now available from Florida (Paul, 1968) and there is a recent sight record from the Virgin Islands (Erdman, 1970). The records from St. Vincent seem to be the most southern in the western Atlantic, and the only West Indian ones supported by specimens. Mention of this species in the St. Vincent fishery was made by Caldwell and Caldwell (in press).

Stenella, SPECIES A—LONG-SNOUTED OR SPINNER DOLPHIN

The Caldwells have color photographs of the head of a specimen of unknown size and sex taken in the fishery on 17 May 1968. The skull (SV-1-SL) was saved but is split longitudinally (see *Tursiops* account above).

The Caldwells measured three females (177.0 [SV-2-SL], 166.5 [SV-3-SL] and 150.0 [SV-4-SL] cm from tip of upper jaw to fluke notch) which were taken on 24 May 1968. They have black and white photographs (Figure 1) of the middle-sized animal taken from several angles, and the skulls of the two largest.

There is confusion in the literature regarding the systematics of the long-snouted spinning dolphins of the genus *Stenella*, and the group is badly in need of revision. The St. Vincent dolphins clearly spin, as observed by all of us at sea off the island, and our specimens (externally) and their skulls compare favorably with those reported from the northern Gulf of Mexico as *S. longirostris* (Gray) by Layne (1965), but neither we nor Layne made similar comparisons with *S. roseiventris* (Wagner) which Rice and Scheffer (1968) retained as a species separate from *S. longirostris* although many writers consider them to be synonyms. For the present we do not apply a specific name to our material but note only that the specimens from St. Vincent appear to belong to the "*longirostris-roseiventris*" group of *Stenella*.

The closest western Atlantic records for dolphins of this type are from the Bahamas near Miami, Fla., to the north (Moore, 1953) and (as *Delphinus microps*) from Brazil (no locality) to the south (Gray, 1850: 126).

Stenella, SPECIES B—SPOTTED OR BRIDLED DOLPHIN

The Caldwells measured a 172.5-cm male taken in the fishery on 24 May 1968, and have black and white photographs (Figure 2) taken from several angles and the skull (SV-1-SF). The photographs, taken under adverse lighting conditions, do not show the spotted pigmentation pattern of the specimen because in addition

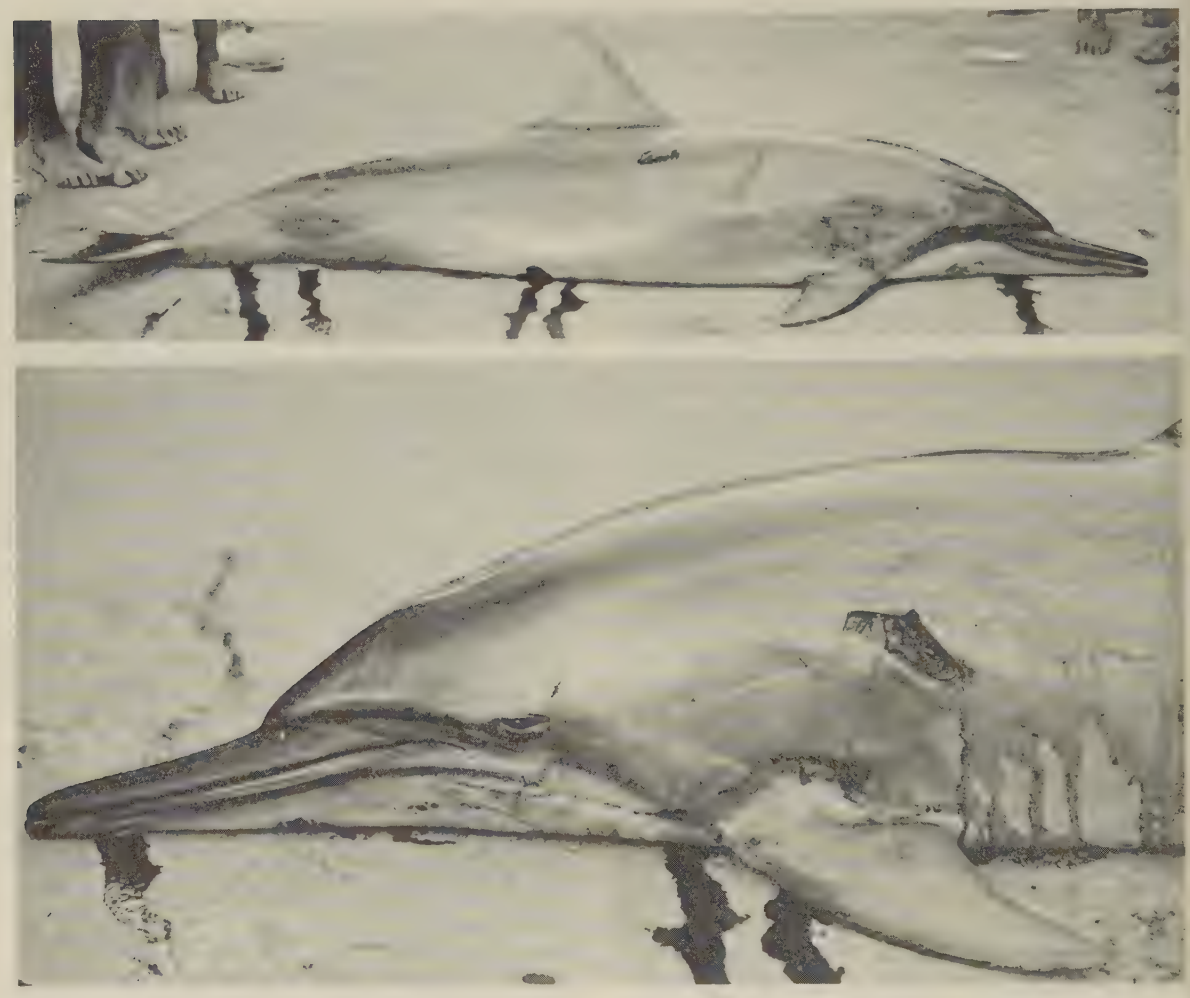

FIGURE 1.—*Stenella*, species A, 166.5-cm female spinner dolphin (SV-3-SL) landed at Barrouallie, St. Vincent, on 24 May 1968. UPPER: lateral view of entire carcass; LOWER: view of head and pectoral region showing prominent features of pigmentation. Photographs made under conditions of adverse lighting several hours after the animal had been harpooned and kept in the sun in an open boat at sea. (Photographs by William A. Huck.)

it had been in the sun most of the day and had turned essentially black as dolphins often do under such circumstances. The underlying spotted pigmentation was, however, like that in the photographs noted below.

The Caldwells also have a color lateral photograph of the anterior part of the body and one of most of the ventral side of a male, apparently an adult (SV-2-SF), taken in early June 1967. Both of these photographs show details of the spotted pigmentation, and a black and white reproduction of the one of the head clearly shows this (Figure 3).

The St. Vincent spotted dolphins we have seen seem best to fit Fraser's (1950b) and Nishiwaki's (1965) discussions and illustrations of *S. frontalis* (G. Cuvier). We tentatively would assign the St. Vincent records to that species were it not better to refrain from doing so at this time because of the already chaotic taxo-

FIGURE 2.—*Stenella*, species B, 172.5-cm male spotted dolphin (SV-1-SF) landed at Barrouallie, St. Vincent, on 24 May 1968. Photograph made under conditions of adverse lighting several hours after the animal had been harpooned and kept in the sun in an open boat at sea. (Photograph by William A. Huck.)

nomic situation in which one finds the spotted dolphins of this genus.

The Caldwells have had considerable experience in Florida with carcasses and live specimens of the spotted species *S. plagiodon* (Cope) (see D. K. Caldwell and M. C. Caldwell, 1966) and do not believe that that species as they understand it is the same as the St. Vincent form. Perrin (1970) concurred that *S. plagiodon* is separable from *S. frontalis* at least on the basis of color pattern. We concur with Mitchell (1970: pl. 5) that the spotted dolphin he pictured as having been taken from continental shelf waters near Trinidad is best assigned to the species *S. plagiodon*. Despite the relatively close proximity of the Trinidad record to St. Vincent (some 275 km), we believe that two species of spotted dolphins are involved and that Mitchell's record bears out an earlier contention by the Caldwells (D. K. Caldwell and M. C. Caldwell, 1966: 2) that *S. plagiodon* is a species found primarily in offshore waters near continents. Around the seemingly more isolated noncontinental islands of the Antilles, at least, it appears in our experience to be replaced by *S.* cf. *frontalis* or some very similar spotted species. We believe, therefore, that Rice and Scheffer (1968) were too conservative in their conclusions regarding spotted dolphins and that more than one species exists.

A mandible, reported as *Prodelphinus* sp., but probably of the same species as ours from St. Vincent, was listed from nearby Barbados by Turner (1912: 138). The next closest western Atlantic record (as *Prodelphinus froenatus*) is from southeastern Florida (Allen, 1925) to the north. It apparently has not been recorded to the south (see Hershkovitz, 1966: 36).

Feresa attenuata GRAY— PYGMY KILLER WHALE

An adult skull (SV-1-FA) of undetermined sex was obtained from the fishery in the spring of 1969. A detailed report on this specimen was prepared (Caldwell and Caldwell, 1971) as it then was the first record for the western Atlantic. After this report was accepted for publication, a record from Texas was published (James, Judd, and Moore, 1970). To our knowledge the St. Vincent specimen is still the only record from the Caribbean and West Indian region.

FIGURE 3.—*Stenella*, species B, apparently adult male spotted dolphin (SV-2-SF) landed at Barrouallie, St. Vincent, in early June 1967. Lateral view of head showing prominent features of pigmentation not shown (but present) in the animal depicted in Figure 2. Animal in freshly killed condition. (Photograph by John R. Sullivan.)

Pseudorca crassidens (OWEN)— FALSE KILLER WHALE

The Caldwells have two adult skulls, a male (SV-1-PC) and a female (SV-2-PC), from animals of unstated size taken in the fishery on 10 September 1970.

Three individuals of this species were taken in the fishery on 9 March 1969, and the Caldwells have several teeth (SV-3-PC) said to have come from one or more of these animals. Five others had been taken on 12 February and seven more were obtained on 7 December 1969.

Except for a brief mention by Caldwell and Caldwell (in press), false killer whales have not been reported from St. Vincent previously. The closest record based on a specimen is from Aves Island off the Venezuelan coast some 675 km to the southwest (Miller, 1920). Bruyns (1969) included a sight record made at sea 115 km east of Tobago (some 280 km southeast of St. Vincent).

Globicephala macrorhyncha GRAY—SHORT-FINNED PILOT WHALE OR BLACKFISH

It is upon this species that the St. Vincent whale fishery is based and it is therefore by far the most abundant species in the overall landings in the fishery. From the fishery we have eight skulls: two from males measuring about 5 (SV-1-GM) and 6 (SV-2-GM) m in total length collected in the second week of June 1967; two from females measuring about 4.5 (SV-3-GM) and 5 (SV-4-GM) m collected with the males; and four others (SV-5 to 8-GM) from adults or near adults of unknown size and sex collected in the summer of 1968.

We have seen many carcasses of fresh-caught animals and have various color and black and white photographs in our files. We also have examined many skulls on the St. Vincent beaches where the carcasses are butchered. The carcasses all have short pectoral flippers and the skulls have expanded premaxillary bones covering the maxillaries. These characters are typical of this species (see Fraser, 1950a).

A female taken on 20 May 1968 contained a male fetus measuring 69 cm in length in a straight line from the anteriormost part of the head to the fluke notch. The Caldwells did not have facilities to preserve this specimen, but have a color photograph (SV-9-GM) which shows it to be a light reddish brown.

A male measuring 4 m in total length that was taken on 21 May 1968 had several of the obligate cetacean barnacle (*Xenobalanus globicipitus*) on the trailing edge of its left flipper.

We have not examined stomach contents of pilot whales landed at St. Vincent, but the whalemen tell use that they include both squid (including very large ones) and fish.

Cyamid parasites and remoras have been observed on blackfish landed at St. Vincent but no specimens so far have been collected.

Blackfish previously have been recorded from or near St. Vincent by Brown (1945, 1947), Hickling (1950), Fenger (1958), Morice (1958), Caldwell and Erdman (1963), Allen (see footnote 5), Morris (see footnote 6), Jackson (see footnote 7), Rathjen and Sullivan (1970), Caldwell and Caldwell (in press), and others. Although specific identifications were not always given, our experience has shown that only *G. macrorhyncha* likely is involved.

Obtained from the Barrouallie Fishermen's Cooperative Society, catch statistics for the period 1962-1970 are included in Table 1. These are pilot whales landed at Barrouallie, the main whaling port of St. Vincent. The monthly var-

TABLE 1.—Landings of blackfish (*Globicephala macrorhyncha*) at the port of Barrouallie, St. Vincent, for the period January 1962, through December 1970. Precise data concerning the following variables, in part affecting the numbers of blackfish landed, are not available: weather conditions, seasonal holidays, numbers of whaling boats operating that month, and more recently, the presence or absence of engines.

Month	1962	1963	1964	1965	1966	1967	1968	1969	1970	Total	Average
January	6	9	8	15	8	6	28	4	10	94	10.4
February	7	15	37	18	5	35	85	12	15	229	25.4
March	11	32	17	19	7	15	12	6	12	131	14.6
April	6	32	42	12	46	24	49	12	21	244	27.1
May	5	57	9	22	50	41	40	53	55	332	36.9
June	15	80	48	12	41	53	21	50	28	348	38.7
July	7	70	11	18	7	21	25	12	27	198	22.0
August	2	31	20	27	12	14	48	(1)	5	[2]159	[2]19.9
September	4	55	43	24	67	16	27	(1)	22	[2]258	[2]32.3
October	4	33	26	9	30	24	44	(1)	14	[2]184	[2]23.0
November	25	10	7	4	40	17	2	24	23	152	16.9
December	5	1	7	3	10	3	6	3	0	[2]38	[2]4.8
Total	97	425	275	183	323	269	387	[3]176	232	2367	

[1] No records kept, but blackfish taken.
[2] Based on landings for 8 years only.
[3] Year incomplete.

iations reflect local weather conditions which affect the ability of the boats to go out and the whalemen to see their quarry, more than the true abundance of whales. In addition, whaling usually almost or completely ceases for approximately 2 weeks before and 2 weeks after Christmas, in observance of the holidays. We are told that the blackfish are there year round.

Orcinus orca (LINNAEUS)—KILLER WHALE

On 13 May 1968, two females (an adult [SV-1-OO] and subadult [SV-2-OO] of unknown sizes) and one juvenile male (SV-3-OO of unknown size) were harpooned from a pod of six animals. The young male was harpooned first, and the other animals reportedly stood by (see M. C. Caldwell and D. K. Caldwell, 1966, regarding this kind of behavior) just as the St. Vincent whalemen report the blackfish in that fishery often do when certain ones of their kind are harpooned. We have several color photographs of the carcasses which show the striking black and white color pattern characteristic of this species. The skulls of the three harpooned specimens are now in the possession of the Caldwells.

Sullivan noted that one of the larger animals had several cyamid parasites on the skin, but specimens were not collected. His photographs show only that the parasites are cyamids but as Leung (1967) did not include *Orcinus* among the known hosts for cyamids the observation is noted. Caldwell and Caldwell (1969) reported that all three of these whales had eaten leatherback sea turtle (*Dermochelys coriacea*).

Three additional killer whales, including a 6-m male with very heavily worn teeth, were taken from a school of eight on 11 July 1968, but no specimens or photographs are available. One of the animals reportedly had cyamid parasites. The school was said to have been moving north about 10 km off the lee shore of the island. The fishermen's records show that four more were taken on 4 June 1969.

Jackson (see footnote 7) noted that killer whales are taken in the St. Vincent fishery and included photographs of carcasses being butchered on shore. Rathjen and Sullivan (1970) and Caldwell and Caldwell (in press) also noted the occasional capture of individuals of this species by the St. Vincent whalemen.

These records are the southernmost in the western North Atlantic for killer whales. Moore (1953) listed this species from off Miami, and Backus (1961) from the Bahamas. Erdman (1970) included several sight records from the general vicinity of the Virgin Islands. In the western South Atlantic, killer whales have not been reported north of Buenos Aires (as the type of *Orca magellanica* Burmeister—see Hershkovitz, 1966: 84).

Physeter catodon (LINNAEUS)—SPERM WHALE

The Caldwells have the prepared lower jaw (SV-1-PCA) and color photographs of the entire carcass of a juvenile female (ca. 8 m in total length) taken in the fishery on 23 May 1968. The teeth were just beginning to erupt and their pulp cavities were completely open. Mention of the stump of the left pectoral flipper of this animal, possibly missing as the result of a shark bite, was made by Wood, Caldwell, and Caldwell (1970), and a photograph of the carcass on the beach was included by Caldwell and Caldwell (in press).

The Caldwells have partial sets of mandibular teeth from two other sperm whales taken in the fishery prior to 1968. In one set (SV-2-PCA) the pulp cavities are fully open, while in the other set (SV-3-PCA), actually smaller teeth, the cavities are fully closed.

The fishermen's records show the capture of three sperm whales on 19 April 1967, two on 2 January 1969, and two on 25 April 1969 (a third was harpooned on this latter occasion but was lost with the boat). Mr. Griffith Arrindell, a leader of the St. Vincent whale fishery, told us that sperm whales are seen most commonly in the region from October to late spring, although some appear to be present year round.

Jackson (see footnote 7) and Rathjen and Sullivan (1970) mentioned that sperm whales are sometimes taken in the St. Vincent fishery and the latter writers included a photograph of the head of an 8-m male. Townsend (1935: chart B) showed sperm whales between St. Vincent and Barbados in January and February, Brown (1942) noted that a few sperm whales once were taken off the lee (western) side of Barbados (toward St. Vincent) and Brown (1945) and Fenger (1958: 44) recorded the fact that this species sometimes is taken in the nearby waters of Bequia and other islands of the Grenadines. Clark (1887: pl. 183) showed active sperm whaling grounds, fished by New England whalers, all around the southern Lesser Antilles in 1880, and this whaling continued thereon into the first part of the 20th century.

Ziphius cavirostris G. CUVIER—GOOSE-BEAKED WHALE OR CUVIER'S BEAKED WHALE

The Caldwells have color photographs of the head of a female (SV-1-ZC) and somewhat longer views of the carcass of her nearly term fetus calf (SV-2-ZC) of undetermined sex taken in the fishery in late summer 1967. Although it was recently reported from nearby Barbados (Caldwell, Rathjen, and Caldwell, in press), this species has not been reported previously from St. Vincent.

UNRECORDED BUT EXPECTED SPECIES

Both the descriptions given us by the whalemen and distributional records from other localities in the region lead us to expect that several additional species eventually will be recorded from St. Vincent. It is beyond the scope of the present preliminary report to discuss these, but a summary of all known records of marine mammals from the West Indies and Caribbean that is in preparation by the Caldwells suggests that the list from St. Vincent might be expected to include any of several species of *Balaenoptera, Stenella caeruleoalba, Delphinus,* and *Mesoplodon. Kogia* and *Peponocephala* might also be expected, but the present suggestive evidence is not as strong.

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CONTRIBUTIONS TO THE BIOLOGY OF THE ROYAL RED SHRIMP, *Hymenopenaeus robustus* SMITH[1]

WILLIAM W. ANDERSON[2] AND MILTON J. LINDNER[3]

ABSTRACT

The royal red shrimp, *Hymenopenaeus robustus*, has been located in commercial concentrations in three areas off the coast of the United States in depths from about 250 to 550 m: one area, known as the St. Augustine Grounds, is off the east coast of Florida; another is off the Dry Tortugas; and the third is off the Mississippi River Delta.

Information on the biology of the species on the St. Augustine Grounds was collected intermittently from 1957 to 1967.

The reproductive systems of males and females are described and illustrated. The ovaries of ripe females are dark red or maroon, and the exceedingly large spermatophores are bright yellow. We observed no indication of sex reversal.

Burrowing and swimming habits as observed from the research submarine *Aluminaut* are summarized.

The early life history of *H. robustus* is unknown. Neither larval nor postlarval stages were encountered in the plankton collections of the M/V *Theodore N. Gill*. Juveniles under 50 mm total length were not caught.

Size of shrimp was not correlated with depth but appeared to be correlated with latitude. Usually shrimp were larger north of lat 29°39′ N than between lat 29°00′ and 29°39′ N.

Males mature at about 125 mm and females at about 155 mm total length. In each sex, maturity is reflected by a change in the regression of carapace length on total length.

Spawning probably occurs throughout the year, but the peak is between January and May.

Year classes are evident in the length distributions. Recruitment on the fishing grounds begins when the shrimp are approaching 1 year of age and are less than 100 mm total length. They reach maturity at about 3 years, and minimum life span appears to be no less than 5 years. Recruitment is probably not complete until at least 2 years. Most of the shrimp on the fishing grounds are mature.

The royal red shrimp, *Hymenopenaeus robustus* Smith, a large deepwater penaeid (Figure 1), has a wide distribution from the east coast of the United States to well down the east coast of South America, principally in depths of 256 to 549 m (140-300 fm).

Surveys by the Bureau of Commercial Fisheries (now the National Marine Fisheries Service) have indicated three major concentrations of these shrimp off the coast of the United States that have commercial possibilities: (1) off St. Augustine on the east coast of Florida (Figure 2), (2) off the Dry Tortugas in the Florida

FIGURE 1.—Adult female and male royal red shrimp, showing great difference in size of sexes. Female (upper) 210 mm and male (lower) 160 mm total length.

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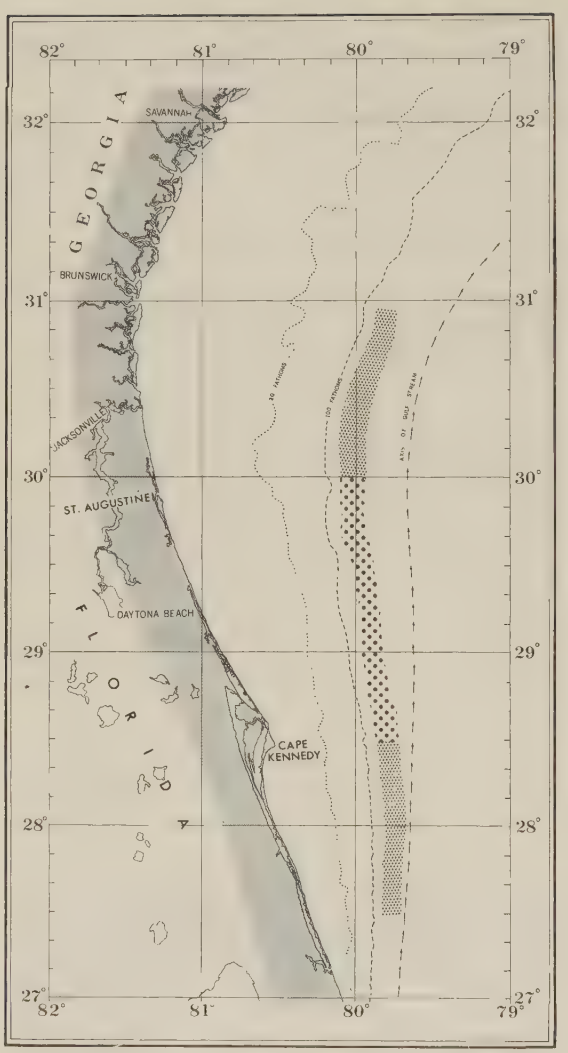

FIGURE 2.—Royal red shrimp grounds off east coast of Florida. Large dots, lat 28°30′ to 30°00′ N, represent most productive portion.

Straits, and (3) off the Mississippi River Delta. Accounts of these surveys were given by Springer and Bullis (1952, 1954), Bullis (1956), Bullis and Rathjen (1959), Bullis and Thompson (1959), Cummins and Rivers (1962), and Bullis and Cummins (1963). Anderson and Bullis (1970) gave an account of direct observations made on the St. Augustine grounds during a dive with the research submarine *Aluminaut* on September 21, 1967; Klima (1969) gave the length-weight relation; and Roe (1969) summarized the distribution on the three major grounds off the southeastern United States.

Biologists at the former BCF Biological Laboratory, Brunswick, Ga., studied the biology of royal red shrimp at the grounds off St. Augustine, Fla., by accompanying vessels of the BCF Exploratory Fishing and Gear Research Base, Pascagoula, Miss., and of the Exploratory Fishing Station, Brunswick, Ga. The work continued from 1957 to 1967, as opportunities arose, on the exploratory fishing vessels *Combat, Silver Bay,* and *Oregon.*

This paper presents the results of these 11 years of intermittent data-gathering.

THE ST. AUGUSTINE GROUNDS

The part of these grounds generally fished extends from about lat 28°30′ N to 30°00′ N in 256 to 475 m (140-260 fm) but the most productive part is between lat 29°00′ N and lat 30°00′ N, and it is from this area that most of the data were obtained.

The BCF surveys of the St. Augustine Grounds indicate that they average about 15.4 km (8.3 nautical miles) in width and have a steep angle of descent. The bottom between 183 and 256 m (100 and 140 fm) is largely untrawlable owing to dense stands of deep-sea alyconarians (sea fans) and limestone formations. Between 256 and 475 m (140 and 260 fm) the bottom is largely sand or silty-sand sediments (referred to as "green mud" by fishermen), is relatively free of obstructions, and provides excellent trawling conditions. Deeper than 475 m (260 fm), extensive patches of deep-sea coral, *Lophelia prolifera,* make the bottom hazardous to trawling with standard shrimp gear.

Anderson and Bullis (1970) made the following observations from the *Aluminaut* in 457 to 274 m (250-150 fm), and between lat 29°10′ and 29°20′ N: "The bottom was remarkedly free from obstructions and consisted of a grayish, loosely constituted sediment that readily clouded the water at the least disturbance. It was

formed into a myriad of shallow depressions and mounds, pitted with holes. Claws protruding from many of these holes indicated the richness of the crustacean bottom fauna. Some fishes were also observed in holes, but these were not nearly so numerous. From the bottom port it was possible to see directly into some of the holes, and we observed animals that would have been invisible at an angle."

These fishing grounds are directly under the Gulf Stream.

DATA AND METHODS

The data were obtained from operations of several vessels either owned or leased by the BCF—M/V *Combat*, a 30-m converted minesweeper; M/V *Silver Bay*, a 29-m New England-type trawler; and the R/V *Oregon*, a 30-m trawler. Bullis (1956) and Bullis and Rathjen (1959) have given details of vessels, gear, and operating procedures.

The data consist of length measurements and observations on ovarian development taken from random samples (100 specimens, if this number or more were caught; the total catch if fewer were caught) at irregular intervals from 1957 to 1967 (Table 1). They also consist of one set of total length-carapace length measurements from nonrandom samples taken during July 1957. Date, location, depth (with two exceptions), and duration of haul are known for each trawling station from which the samples were taken. Data on bottom temperatures are not available. The location of each trawling station was obtained by sonar fixes and the depth, in fathoms, from sonic depth recordings. The depths have been converted to the nearest meter.

TABLE 1.—Cruise dates, number of stations, and number of *H. robustus* in samples.

Vessel	Cruise dates	Stations sampled	Shrimp measured	
			Males	Females
		Number	*Number*	*Number*
Combat	Apr 26-28, 1957	7	495	624
Combat	May 29-31, 1957	3	187	373
Combat	July 17-30, 1957	9	350	456
Combat	Aug. 13-20, 1957	18	798	874
Silver Bay	Nov. 20-25, 1957	14	441	755
Silver Bay	June 11-22, 1958	28	1,136	1,811
Silver Bay	Jan. 18-28, 1960	13	408	356
Silver Bay	Apr. 30-May 3, 1960	10	379	463
Silver Bay	Apr. 28-May 1, 1961	12	489	601
Silver Bay	Jan. 16-Feb. 22, 1962	24	881	1,386
Silver Bay	Aug. 22-28, 1962	10	393	525
Silver Bay	Sept. 25-28, 1962	8	373	376
Silver Bay	Feb. 5-7, 1964	6	290	309
Oregon	Nov. 11-18, 1964	21	863	1,233
Oregon	July 20-22, 1967	6	251	299
Total		189	7,734	10,441

Throughout almost every cruise during which length measurements were taken, the operation simulated commercial fishing; consequently the shrimp measured were caught by several types of trawls in a wide range of sizes. Mesh sizes, however, probably did not vary much. The most commonly used trawl was constructed of "commercial" nylon webbing with 2-inch (50.8-mm) stretched mesh in the body and 1½-inch (38.1-mm) stretched mesh in the cod end. These sizes of mesh are generally used by commercial shrimp fishermen. As shown by Berry and Hervey (1965), the greatest inside dimensions usually are somewhat less than 50.8 mm and 38.1 mm, respectively. Measurements we made on the greatest inside mesh dimensions of the shrimp trawls used by the exploratory vessels for capturing *H. robustus* indicate a range between 43 and 48 mm (mean 45.7 mm or 1.8 inches) for the "commercial" 50.8-mm mesh and between 30 and 34 mm (mean 31.7 mm of 1¼ inches) for the "commercial" 38.1-mm mesh.

Mesh selectivity studies have not been made on *H. robustus*, but we doubt that the variability in types and sizes of trawls appreciably affected the lengths of shrimp caught. We assume that mesh selectivity would be about the same as that shown by Berry and Hervey (1965) for *Penaeus aztecus* for 2-hr tows. Most of our tows lasted 2 to 4 hr, with 3-hr tows predominating. The 50% escapement length for 31.7-mm mesh would thus be about 70 mm total length and specimens of *H. robustus* less than 50 mm total length, if present, would have been represented in the catches. We can expect, however, that specimens less than about 100 mm total length probably were not represented in their true perspective in the random samples.

Total length measurements (tip of rostrum to end of telson—all length measurements are

total length unless otherwise indicated) were made in ½-cm units on a measuring board so adjusted that when the ½-cm units were converted to millimeters the midpoints fell on 3 and 8 (e.g., a length of 25 ½-cm units represents lengths between 121 and 125 mm with midpoint at 123 mm; similarly, the midpoint in millimeters for 26 ½-cm units is 128 mm). Carapace lengths (orbital angle to mid-dorsal end of carapace) were measured with calipers to the nearest millimeter. Both total length and carapace length measurements were made of freshly caught specimens.

The ovarian stages, determined by visual inspection at the time each female was measured, were based on size and color of the ovaries. We also noted whether spermatophores were attached to females.

SYSTEMATICS

In the shrimp family Penaeidae the royal red shrimp, *Hymenopenaeus robustus* Smith, belongs in the subfamily Solenocerinae, which is distinguished from the three other subfamilies by having a postorbital spine (Figure 3).

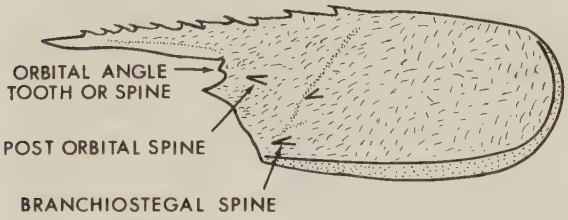

FIGURE 3.—Outline of carapace of *H. robustus* showing position of spines.

Three genera (*Haliporus* Bate, *Hymenopenaeus* Smith, and *Solenocera* Lucas) make up the subfamily Solenocerinae. *Solenocera* is distinct from the other two genera in having the antennular flagella flattened or hollowed out—channellike in structure—rather than cylindrical and filiform. *Hymenopenaeus* has a single pair of lateral telson spines and lacks podobranchs behind segment VIII, whereas *Haliporus* has several pairs of lateral telson spines and podobranchs posterior to segment VIII.

Within the genus *Hymenopenaeus*, three species (*H. robustus, H. modestus* Smith, and *H. lucasii* Bate) are separated from all other species by the following combination of characters: branchiostegal spine present, pterygostomian spine absent, and no postrostral teeth separated from the rostral group (Figure 3).

H. robustus is distinct from *H. modestus* and *H. lucasii* in having a tooth or spine in the orbital angle (Figure 3).

BIOLOGY OF THE SHRIMP

REPRODUCTIVE SYSTEMS

Internal

The internal reproductive organs of royal red shrimp are so similar to those described by Angelescu and Boschi (1959) for *Hymenopenaeus muelleri* and by King (1948) and Young (1959) for the white shrimp, *Penaeus setiferus*, that only gross anatomy is given here.

The ovaries are paired. In the cephalothoracic region they are partly fused; each ovary has an anterior pointed lobe and 6 to 8 fingerlike lateral projections which lie over the hepatopancreas. A lobe from each ovary extends nearly the full length of the abdomen dorsolateral to the intestine. The oviducts lead to genital pores at the bases of the third pereiopods (Figure 4).

The testes are also paired and occupy a position in the cephalothoracic region similar to that of the ovaries. Each testis has several lateral lobes, and a looped vas deferens which connects to the terminal ampoule on the coxa of the fifth pereiopod. The testes do not extend into the abdomen.

External

Details of the structure of the thelycum in the female (considered to be modifications to the sternal plates of somites XII, XIII, and XIV) are shown in Figure 4. Note the bristly, cuplike paired openings of the oviducts at the bases of the third pereiopods; the rectangular plate with

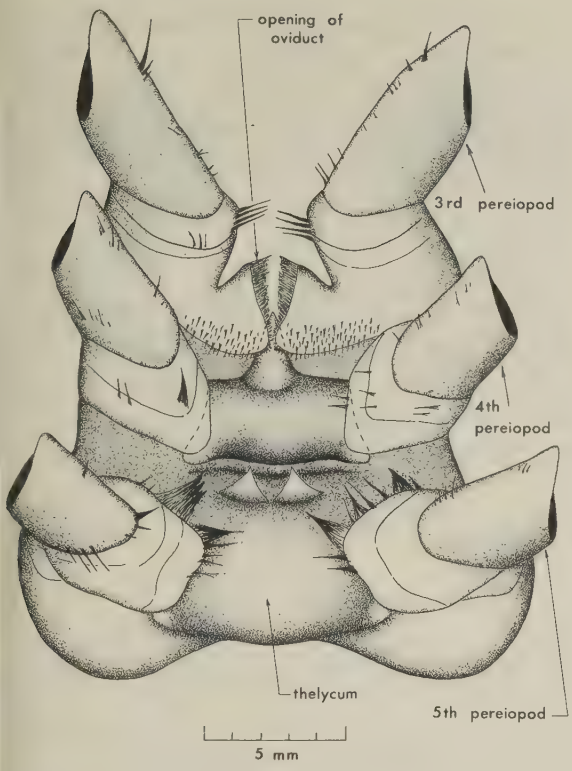

FIGURE 4.—Ventral view of thorax of adult female.

a forward projecting cone-shaped protuberance which lies between the fourth pereiopods; the paired triangular protuberances about midway between the fourth and fifth pereiopods; and the dome-shaped area between the fifth pereiopods.

Burkenroad (1936) described in detail the petasma of *H. robustus* but did not illustrate it. Figure 5 shows the petasma of *H. robustus*, spread open to show its structure.

Spermatophore

Compared with the spermatophores of the white shrimp (*Penaeus setiferus*), the brown shrimp (*P. aztecus*), and the pink shrimp (*P. duorarum*)—all penaeid shrimp similar in size to *H. robustus*—the spermatophore of the royal red shrimp is exceptionally large. In fresh material the spermatophore is bright yellow.

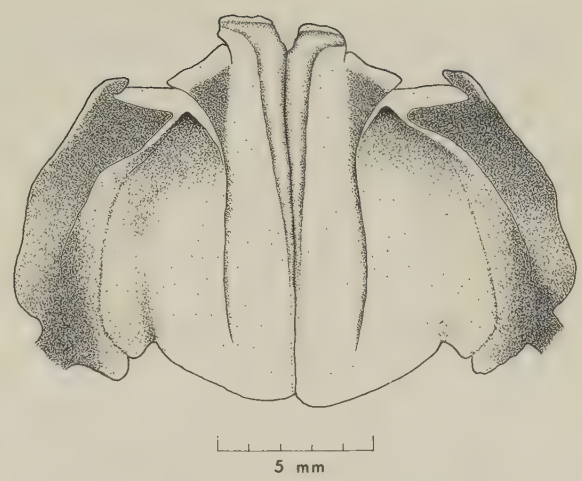

FIGURE 5.—Petasma of male spread open to show arrangement of rods and folds.

Figure 6 shows a spermatophore in attached position; Figure 7 shows a detached spermatophore. The winglike protuberances extend between the pereiopods, and the knobby and bristly sections of the coxae help hold the spermatophore in place. A gluelike substance that accompanies the spermatophore when attached by the male also helps hold it secure. The spermatophores of royal red shrimp are much more securely attached than those of the white shrimp and are not easily dislodged.

HABITS

Anderson and Bullis (1970) contributed most of our limited knowledge of the habits of this deep-sea shrimp. Their observations from the submarine *Aluminaut* were as follows: "Bottom photographs had previously indicated that royal-red shrimp stayed on the sea-floor surface, but we saw numerous shallow furrows (1 to 3 ft long) in the bottom in which royal-red shrimp were partly buried. They apparently do not burrow as deeply or completely as do brown and pink shrimp. We believe the shrimp plow into the bottom in search of food rather than protection, and that this feeding activity produces the grooves or furrows.

"When disturbed, the royal-red shrimp rise gently from the furrows and swim in normal

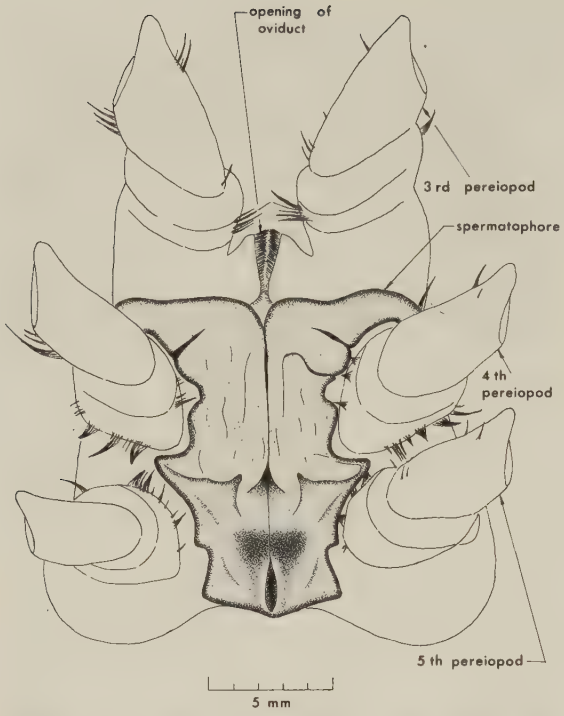

FIGURE 6.—Ventral view of thorax of adult female with spermatophore (shaded) attached.

upright position. If frightened, they flip back in typical penaeid fashion by quick flexing of the abdomen and then swim forward rapidly, but usually they are turned on their sides so that they bounce off the bottom every few feet. At the end of the run they stand on the bottom rather than burrow in. The shrimp walk either forward or sideways. Color varied from grayish pink to red—similar to colors observed on trawl-caught specimens."

LARVAL, POSTLARVAL, AND JUVENILE STAGES

The larvae of *Hymenopenaeus robustus* are unknown. Several attempts were made by Anderson to hatch eggs from ripe females bearing spermatophores. The eggs failed to develop, however—perhaps because of the drastic changes in temperature and pressure when the animals were quickly brought from the cold

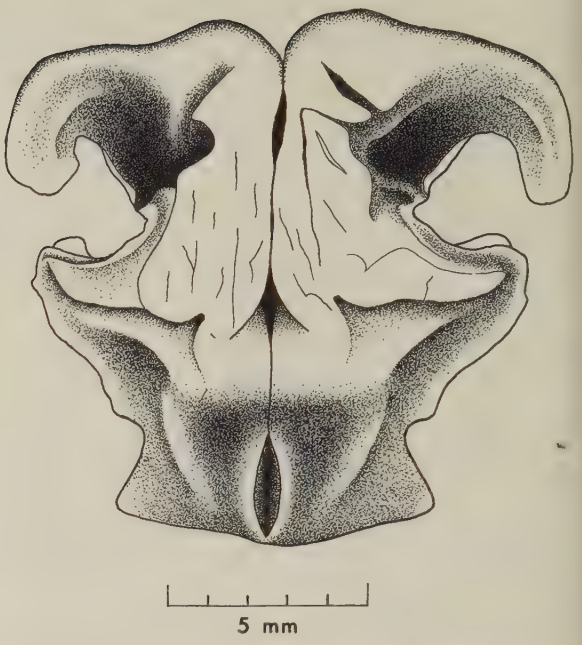

FIGURE 7.—Ventral view of detached spermatophore.

waters of about 385 m (200 fm) to the warm surface waters of the Gulf Stream.

In attempts to find larval and postlarval royal red shrimp, we examined numerous plankton samples from the M/V *Theodore N. Gill* cruises, covering all seasons, in an area from about the 183-m (100 fm) contour to well beyond the axis of the Gulf Stream and over the entire length of the St. Augustine Grounds. Only a few larval or postlarval penaeids were found and only one of these, a *Solenocera*-like mysis stage, was considered as possibly being *Hymenopenaeus* (Harry L. Cook, then a fishery biologist at the BCF Biological Laboratory, Galveston, Tex., kindly made the identifications).

Burkenroad (1936) has provided the only record of postlarval *H. robustus*. He described nine specimens (all dead when examined), which he believed to be juveniles (postlarvae), that were collected in the northern Gulf of Mexico off the mouth of the Mississippi River. Eight (12.0-21.5 mm total length) were taken at R/V *Atlantis* station 2377 on March 24, 1935, and one (no length given) was taken at *Atlantis* station 2381 on March 26, 1935.

LATITUDE AND DEPTH DISTRIBUTIONS

A preliminary examination of our data suggested that royal red shrimp tended to be larger in the northern part of the collection area than in the central part, and that size was inversely related to depth. Correlations were significant between median lengths of females and latitude ($r = +0.46$; $t = +6.65$; $Y = 23.39 + 0.09X$) and between median lengths of females and depth ($r = -0.46$; $t = -6.65$; $Y = 53.78 - 0.11X$). The data used for these correlations were from the 164 stations at which 20 or more females were measured; the lengths are in ½-cm units. We did not repeat the calculations for the males because when the smaller size groups of females were caught in a tow, invariably the smaller size groups of males also were caught.

For the latitude correlation we grouped the median lengths by 10′ intervals of latitude. A graph of the data showed that female shrimp tended to be smaller between lat 29°00′ and 29°39′ N than between lat 29°40′ and 30°13′ N. We had samples from only five stations south of lat 29°00′ N, of which four were between lat 28°00′ and 28°39′ N. Although the data suggested that large shrimp also tended to inhabit the southern part of the grounds, we are unwilling to draw conclusions for the area south of lat 29°00′ N from this small sample.

The size and latitude relation confirmed reports of the fishermen that usually, but not always, they encountered larger shrimp on the northern portion of the grounds than on the central portion. The fishermen rarely fish the southern part of the grounds, and we received conflicting reports on the size of shrimp caught in this area.

For the depth relation we grouped the data by 25-fm (46-m) depth intervals (151-175; 176-200, etc.). The highly significant negative relation between size and depth (large shrimp in shallow water and smaller shrimp in deeper water) did not agree with some of our data (Table 2) nor with reports from the fishermen and exploratory fishing personnel; hence we suspected that this relation and perhaps that with latitude were fortuitously caused by the fishing pattern at the times our samples were taken. We therefore re-examined the data and selected only those cruises during which more than one depth class was fished in the same latitude zone and those cruises during which the same depth class was fished in the two latitude zones. The depth classes used were 150 to 175 fm (274-320 m), 176 to 200 fm (322-366 m), 201 to 225 fm (368-411 m), and 226+ fm (413+ m). The latitude zones chosen were lat 29°00′ to 29°39′ N and 29°40′ to 30°13′ N. We had no stations south of lat 29°00′N that met the requirements.

TABLE 2.—Median length of *H. robustus* and depth of hauls for eight stations in the same latitude—*Silver Bay* cruise September 25-28, 1962.

Lat N	Depth of hauls		Median length	
			Males	Females
	m	*fm*	*mm*	*mm*
29°54′	324-329	177-180	138	178
29°54′	329-338	180-185	138	178
29°54′	348	190	138	178
29°54′	357-362	195-198	138	178
29°53′	366	200	138	183
29°53′	375	205	138	178
29°53′	384	210	138	173
29°53′	411-421	225-230	138	178

After the selection was made, we had 12 cruises with 93 stations and 2 depth classes that could be compared by latitude zone for the same depth class; and 18 cruises with 95 stations and 4 depth classes that could be compared by depth class for the same latitude zone. Only those stations were selected from which 20 or more females were measured. The results are shown in Figures 8 and 9 and in Appendix Tables 1 and 2.

In Figure 8 we have plotted the mean median lengths (in ½-cm units) as scatter diagrams against depth class with latitude zone lat 29°00′ to 29°39′ N as the abscissa and latitude zone lat 29°40′ to 30°13′ N as the ordinate. If no relation existed between the length of the shrimp and the latitude, the dots and crosses would be scattered along and on either side of the 45° lines. All 12 marks fall above the 45° lines, however, showing that shrimp tended to be larger north of lat 29°39′ N than in the area between lat 29°00′ and 29°39′ N.

In Figure 9 we have plotted the mean median lengths as scatter diagrams separately for each

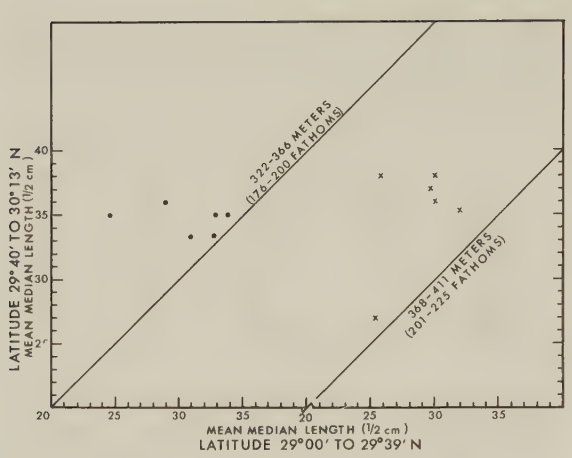

FIGURE 8.—Mean median length of females by latitude and depth (see text for explanation).

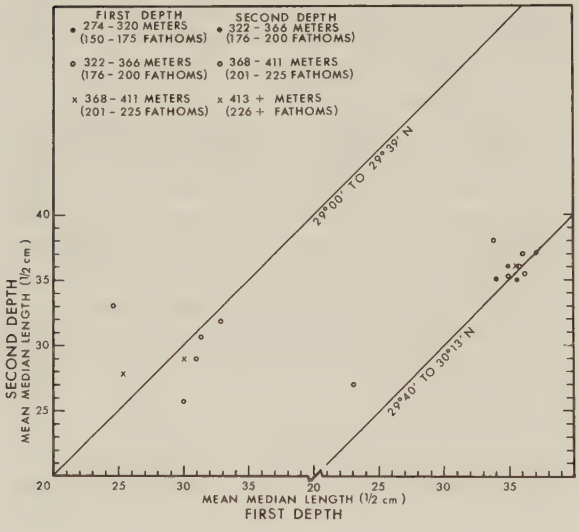
FIRST DEPTH
• 274 - 320 METERS (150 - 175 FATHOMS)
o 322 - 366 METERS (176 - 200 FATHOMS)
x 368 - 411 METERS (201 - 225 FATHOMS)
SECOND DEPTH
• 322 - 366 METERS (176 - 200 FATHOMS)
o 368 - 411 METERS (201 - 225 FATHOMS)
x 413 + METERS (226 + FATHOMS)
29°00' TO 29°39' N
29°40' TO 30°13' N
SECOND DEPTH
MEAN MEDIAN LENGTH (1/2 cm)
MEAN MEDIAN LENGTH (1/2 cm)
FIRST DEPTH

FIGURE 9.—Mean median length of females by depth and latitude (see text for explanation).

latitude zone, with the first or shallowest depth class as the abscissa and the next succeeding depth class as the ordinate. The marks for each latitude zone appear to fall in a random fashion about the 45° lines; consequently there was no apparent relation between the length of the shrimp and the depth from which they were caught. This figure also indicates that the average size of shrimp is usually, but not always, larger in that part of the St. Augustine Grounds north of lat 29°39′ N than between lat 29°00′ and 29°39′ N.

We are not certain why shrimp tend to be smaller in the central than in the northern portion of the St. Augustine Grounds unless most of the recruitment is in the central area. If shrimp also are larger in the southern portion of the grounds (as our meager data from that area suggest), the obvious conclusion is that most of the recruitment occurs in the central portion of the grounds; otherwise, it may be that recruitment is from the south.

SIZE AT MATURITY

The terminal ampoules were small and poorly developed in most males less than about 125 mm long but were large and prominent in specimens greater than this length. This observation suggests that sexual maturity in the males is reached at about 125 mm total length.

Development of the ovaries began in most females at about 136 mm total length, but maturity was not attained until they were about 155 mm long. In the random samples, all of the 1,327 females that had attached spermatophores were ripe. Of this number only 55 or about 4% were less than 151 mm long. The smallest ripe shrimp with attached spermatophores (three individuals) were in the 136- to 140-mm size class.

Lindner and Anderson (1956) demonstrated that female *Penaeus setiferus* underwent certain morphometric changes on attaining maturity. It is evident from Figures 10 and 11 and Appendix Table 3 that the regressions of carapace length on total length for both male and female royal red shrimp also show changes in slope at about the lengths at which each sex reaches maturity. Male royal red shrimp (Figure 10) demonstrate a change in slope at the midpoint of the 126- to 130-mm class interval, which agrees with field observations on the size at which the males reach maturity.

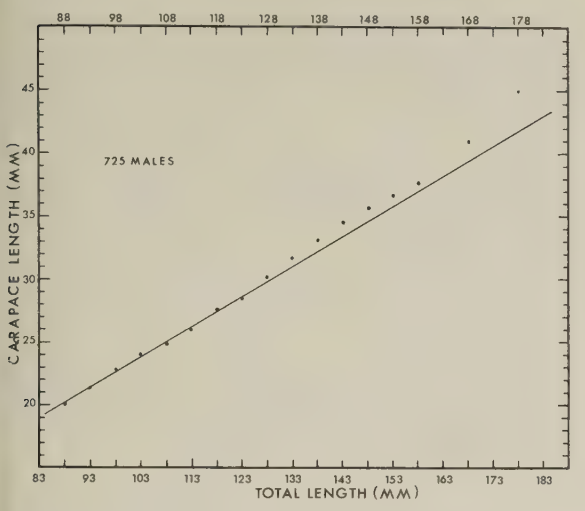

FIGURE 10.—Regression of carapace length on total length for males.

For female *H. robustus* (Figure 11) the break occurs at the midpoint of the 151- to 155-mm class interval and the new slope is not reached until the midpoint of the 161- to 165-mm class interval. Here again this morphometric change is associated with maturity.

These regressions were made from data gathered in July 1957. Possibly the lengths at which the slopes change would be different at other times of the year.

SPAWNING

It was possible to separate several stages of ovarian development in the field without microscopic examination, because maturation is accompanied by changes in size of the ovaries and by very distinct color changes. In the field, however, we were unable to distinguish with certainty the spent females. These were included in the "undeveloped" and "developing" categories. We usually recorded ovary development in the following stages:

1. UN = undeveloped. In this stage the tiny ovaries are almost threadlike and transparent.
2. D = developing. The ovaries have increased markedly in size and are opaque but have not developed a distinctive color.
3. P = pink. The ovaries continue to increase in size and first take on a light yellow color which rapidly becomes light pink.
4. R = ripe. Now swollen to full size, the ovaries are a dark red or maroon color. At this stage the male attaches the spermatophore to the female.

The length distribution of shrimp with pink ovaries differed little from the length distribution of those with ripe ovaries. Because we have no conception of the time required for the pink stage to develop to the ripe stage, and because the sizes were similar, we grouped these two ovarian stages and called them ripe. We also grouped females with undeveloped and developing ovaries, mainly to avoid discarding the data collected during the first two cruises in 1957, when these two stages were not differentiated.

We have presented this material in Figure 12 by seasonal periods. The periods chosen were November; January and February; April, May, and June; and July, August, and September.

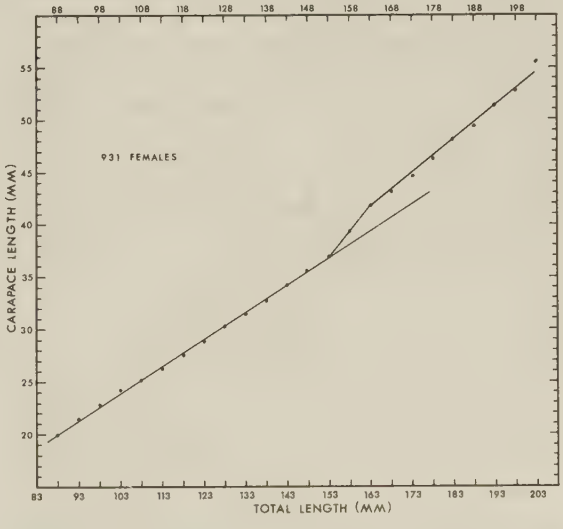

FIGURE 11.—Regression of carapace length on total length for females.

Because many more shrimp were measured during some cruises than during others in the same season, we weighted the data in Figure 12 and Appendix Table 4 to give each cruise equal weight, irrespective of the number of shrimp in the samples. Figure 12 demonstrates that the peak of spawning is during the winter and spring. Spawning probably is not extensive before December and is essentially completed by June, although some spawning continues throughout the year. Figure 12 also indicates that few females less than 150 mm long have ripe ovaries.

The occurrence of small specimens reported by Burkenroad (1936) in March corresponds with our estimate of the peak spawning season.

AGE CLASSES

In compiling total length distributions for males and females, we have again given equal weights to data from each cruise irrespective

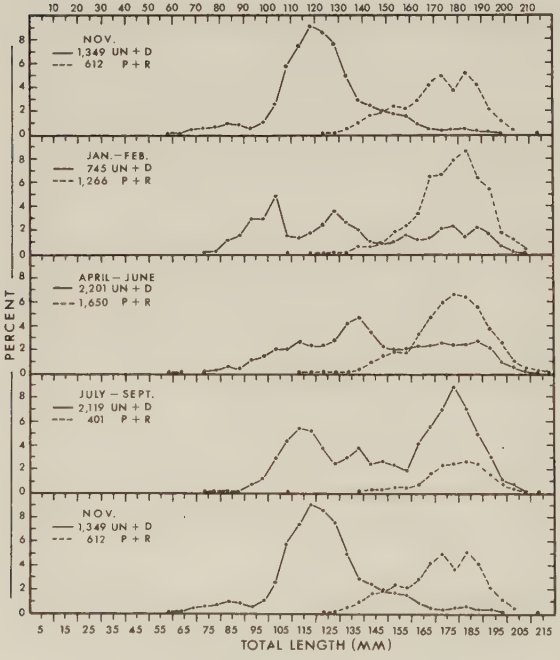

FIGURE 12.—Seasonal length distributions of female *H. robustus* by ovarian stages. (UN = undeveloped; D = developing; P = pink; R = ripe.)

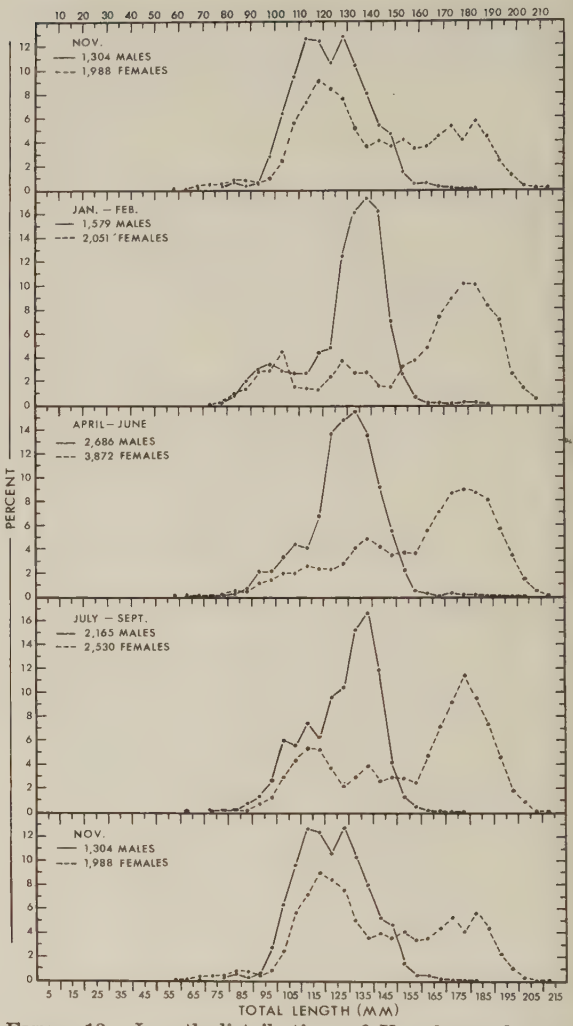

FIGURE 13.—Length distributions of *H. robustus* by sex and season.

of the number of shrimp measured, and we present the data for the same seasonal periods used to show the spawning season (Figure 13 and Appendix Table 5). The relative heights of the modes can be misleading because the data are scattered sparsely through 11 years and the appearance of a dominant year class in one sample (as occurred in November 1964) can have a disproportionate effect when applied to the material as a whole.

The graphs for the females are more readily interpreted than those for the males. In No-

vember the females show a mode at about 80 mm total length that can be followed readily throughout the year to a second mode at about 120 mm. The mode at 120 mm can also be traced throughout the year to the bimodal group with modes at 143 and 153 mm. The bimodality of this latter group we believe to be fortuitous, but it can be followed to a hump between 153 and 158 mm in the January-February distributions. Thereafter the modes become lost in the large group of mature females. We believe that the two additional modes in our November distributions (173 and 183 mm) also are fortuitous and result from the sampling procedures.

A group of small male shrimp also appears in November, forming a mode at about the same length as that for the females, which can be traced throughout the year to the second mode at about 115 mm. This second mode can be traced as a hump to the left of the main mode from January through September to the third mode at 128 mm, beyond which it is lost.

We made various attempts to fit Von Bertalanfy growth curves to the data without satisfactory results other than it was apparent the first two modes could be attributed to 1- and 2-year-old shrimp. Evidently morphometric changes associated with maturity preclude the use of total length as a means of determining age of *H. robustus* after they reach maturity.

We believe the first three groups of males and females we have cited are 3 distinct age classes. If the peak of spawning is in March, both sexes would be about 100 mm total length at 1 year of age. From our data it is impossible to distinguish more than the first 3 age classes. The older age classes, which probably represent 2 or more additional years, would give a minimum life span of 5 years. Probably, however, at least some of the largest shrimp are older than this.

SIZE AND AGE AT RECRUITMENT

When we consider all of our length measurements as a unit, either unweighted, weighted to give each cruise equal weight, or weighted to give each year equal weight, we obtain almost identical distributions. In Figure 14 we show

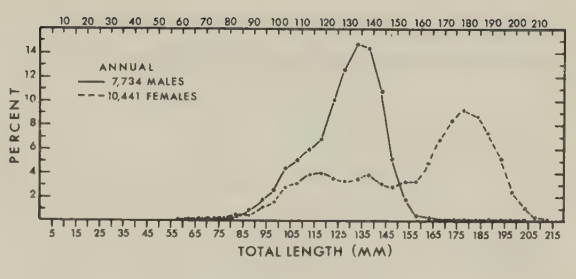

FIGURE 14.—Length distributions of *H. robustus* by sex for all samples combined.

these length distributions weighted to give equal weight to collections during each cruise. It is readily evident from these curves that only the groups presumed to represent the second, third, and fourth and older age groups are present in substantial numbers and that the population is composed largely of mature shrimp.

Although the data are not adequate, they suggest that recruitment starts at about 1 year of age, but the shrimp are not fully recruited until about 2 years of age, and recruitment may not be complete until the shrimp are mature—about 3 years old. In the combined length distributions (Figure 14), 55% of the females were longer than 160 mm and 61% of the males were longer than 125 mm (the lengths at which we believe each sex is fully mature). Only 6% of the males and 4% of the females were less than 100 mm long. The smallest shrimp we sampled was in the 56- to 60-mm length class. As we mentioned earlier, we do not believe that gear selectivity causes the scarcity of shrimp under 100 mm long and the lack of them under 56 mm long. Royal red shrimp do not appear on the fishing grounds at sizes smaller than about 55 mm. The observations of Anderson and Bullis (1970) who had clear visibility of the bottom from a distance of less than 1 m, substantiated the lack of small shrimp on the St. Augustine Grounds. Furthermore, 37 *H. modestus*, 43 to 93 mm long (mode, 63 mm) were taken on April 20, 1957, in 225 fm with a 40-ft flat shrimp trawl fitted with commercial 2-inch stretched mesh in the body and 1½-inch mesh in the cod end. In addition, H. R. Bullis, BCF Pascagoula, Miss. (personal communication), concerning trawling on the *H. robustus* grounds

off the Mississippi River Delta, stated, "Of special interest was the discovery of high densities of small red (*Hymenopenaeus debilis*) shrimp in 208 fathoms. These shrimp averaged less than 35 mm total length." These two species of *Hymenopenaeus* are similar in shape to *H. robustus;* consequently, we believe that small *H. robustus* would have been taken in our gear if they had been present on the fishing grounds. We have no idea where they might be.

The sex ratios for all the data combined show 42.6% males and 57.4% females. Some, but probably not all, of this difference is undoubtedly the result of mesh selectivity. We observed no indication of sex reversal in the species.

Appendix Table 6 shows the length distributions in numbers of shrimp by sex for each cruise.

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APPENDIX TABLES

APPENDIX TABLE 1.—Mean median lengths of female *H. robustus* by depth, latitude, and cruise.

Item	322 to 366 m (176-200 fm)											
	Lat 29°00′ to 29°39′ N						Lat 29°40′ to 30°13′ N					
Cruise dates	Aug. 13-20 1957	Nov. 20-25 1957	June 11-22 1958	Jan. 18-28 1960	Apr. 28-May 1 1961	Jan. 16-Feb. 22 1962	Aug. 13-20 1957	Nov. 20-25 1957	June 11-22 1958	Jan. 18-28 1960	Apr. 28-May 1 1961	Jan. 16-Feb. 22 1962
Number of stations	8	5	3	1	2	2	1	4	22	1	2	1
Mean median length (½ cm)	32.8	30.9	32.7	24.5	29.0	33.8	35.0	33.4	33.6	35.0	36.0	35.0

Item	368 to 411 m (201-225 fm)											
	Lat 29°00′ to 29°39′ N						Lat 29°40′ to 30°13′ N					
Cruise dates	Apr. 26-28 1957	July 17-30 1957	Aug. 13-20 1957	Aug. 22-28 1962	Feb. 5-7 1964	Nov. 11-18 1964	Apr. 26-28 1957	July 17-30 1957	Aug. 13-20 1957	Aug. 22-28 1962	Feb. 5-7 1964	Nov. 11-18 1964
Number of stations	2	2	4	2	4	15	1	1	2	2	1	5
Mean median length (½ cm)	25.8	30.0	31.9	30.0	29.6	25.4	38.0	38.0	35.3	36.0	37.0	27.0

APPENDIX TABLE 2.—Mean median lengths of female *H. robustus* by latitude, depth, and cruise.

Item	Lat 29°40′ to 30°13′ N											
	First depth 274-320 m (150-175 fm)				Second depth 322-366 m (176-200 fm)							
Cruise dates	May 29-31 1957	Jan. 18-28 1960	Apr. 28-May 1 1961	Jan. 16-Feb. 22 1962	May 29-31 1957	Jan. 18-28 1960	Apr. 28-May 1 1961	Jan. 16-Feb. 22 1962				
Number of stations	1	1	6	6	1	1	2	1				
Mean median length (½ cm)	37.0	34.0	34.9	35.6	37.0	35.0	36.0	35.0				
	First depth 322-366 m (176-200 fm)						Second depth 368-411 m (201-225 fm)					
Cruise dates	July 17-30 1957	Aug. 13-20 1957	Aug. 22-28 1962	Sept. 25-28 1962	Feb. 5-7 1964	Nov. 11-18 1964	July 17-30 1957	Aug. 13-20 1957	Aug. 22-28 1962	Sept. 25-28 1962	Feb. 5-7 1964	Nov. 11-18 1964
Number of stations	5	1	3	5	1	1	1	2	2	2	1	5
Mean median length (½ cm)	33.8	35.0	35.7	36.2	36.0	23.0	38.0	35.3	36.0	35.5	37.0	27.0
	First depth 368-411 m (201-225 fm)						Second depth 413 plus m (226 plus fm)					
Cruise dates			Sept. 25-28 1962						Sept. 25-28 1962			
Number of stations			2						1			
Mean median length (½ cm)			35.5						36.0			

Item	Lat 29°00′ to 29°39′ N									
	First depth 322-366 m (176-200 fm)					Second depth 368-411 m (201-225 fm)				
Cruise dates	April 26-28 1957	Aug. 13-20 1957	Nov. 20-25 1957	Jan. 18-28 1960	Apr. 30-May 3 1960	April 26-28 1957	Aug. 13-20 1957	Nov. 20-25 1957	Jan. 18-28 1960	Apr. 30-May 3 1960
Number of stations	2	8	5	1	6	2	4	2	1	4
Mean median length (½ cm)	30.0	32.8	30.9	24.5	31.3	25.8	31.9	29.0	33.0	30.6
	First depth 368-411 m (201-225 fm)					Second depth 413 plus m (226 plus fm)				
Cruise dates		July 17-30 1957		July 20-22 1967			July 17-30 1957		July 20-22 1967	
Number of stations		2		4			1		2	
Mean median length (½ cm)		30.0		25.3			29.0		27.8	

APPENDIX TABLE 3.—Regression of carapace length on total length for *H. robustus*.

Total length	Males	Females	Mean carapace length	
			Males	Females
mm	*Number*	*Number*	*mm*	*mm*
83	--	1	--	20.0
88	6	1	20.2	20.0
93	8	4	21.3	21.5
98	12	5	22.8	22.8
103	22	10	24.0	24.2
108	23	12	24.9	25.1
113	45	18	25.9	26.2
118	63	23	27.5	27.6
123	94	20	28.8	28.9
128	86	17	30.1	30.3
133	104	34	31.7	31.4
138	115	46	33.1	32.8
143	94	53	34.5	34.1
148	34	60	35.6	35.4
153	14	36	36.8	37.0
158	3	36	37.7	39.3
163	--	56	--	41.7
168	1	86	41.0	43.1
173	--	102	--	44.6
178	1	82	45.0	46.3
183	--	77	--	48.1
188	--	81	--	49.4
193	--	49	--	51.3
198	--	16	--	52.9
203	--	6	--	55.5
Total	725	931		

APPENDIX TABLE 4.—Length distributions of ovarian stages by season and cruise.
[UN = undeveloped; D = developing; P = pink; R = ripe]

Total length	Fall				Winter					
	Nov. 20-25, 1957		Nov. 11-18, 1964		Jan. 18-28, 1960		Jan. 16-Feb. 22, 1962		Feb. 5-7, 1964	
	UN + D	P + R	UN + D	P + R	UN + D	P + R	UN + D	P + R	UN + D	P + R
mm	%	%	%	%	%	%	%	%	%	%
53	--	--	--	--	--	--	--	--	--	--
58	--	--	0.2	--	--	--	--	--	--	--
63	--	--	0.2	--	--	--	--	--	--	--
68	--	--	0.7	--	--	--	--	--	--	--
73	--	--	0.9	--	--	--	0.1	--	0.3	--
78	0.1	--	1.1	--	0.3	--	0.1	--	0.3	--
83	0.5	--	1.2	--	2.4	--	0.1	--	0.7	--
88	0.5	--	1.1	--	3.4	--	0.3	--	0.7	--
93	--	--	1.0	--	6.7	--	0.3	--	1.6	--
98	0.7	--	1.2	--	5.3	--	0.2	--	3.3	--
103	2.3	--	2.7	--	8.0	--	0.6	--	5.8	--
108	5.2	--	6.1	--	2.4	0.3	0.7	--	1.3	--
113	5.6	--	9.0	--	1.5	--	0.3	--	2.0	--
118	5.2	--	12.7	--	1.2	--	1.3	0.1	2.6	--
123	3.2	--	13.7	0.2	1.8	--	1.8	0.1	3.6	--
128	2.7	0.1	12.2	0.1	3.2	--	1.0	0.1	6.1	--
133	2.4	0.6	7.3	0.1	2.4	--	1.0	0.1	4.3	--
138	1.5	1.6	4.0	0.1	2.8	0.9	0.5	0.1	2.6	0.7
143	2.1	2.8	2.7	0.4	0.3	0.6	0.3	0.1	2.3	1.0
148	1.0	2.7	2.7	0.9	0.9	1.8	0.7	0.3	1.0	0.3
153	0.8	3.2	2.5	1.4	1.5	3.0	0.5	0.9	1.0	1.6
158	1.5	3.1	1.4	1.0	2.4	3.3	1.4	1.4	1.0	2.3
163	0.4	4.5	1.2	1.1	1.5	4.2	1.9	4.2	0.3	2.0
168	--	7.4	0.7	0.7	0.6	7.5	2.7	8.5	0.7	3.0
173	--	8.5	0.6	1.2	1.2	3.9	4.4	12.4	0.7	3.6
178	0.4	6.5	0.3	0.8	1.5	5.8	3.7	12.9	1.6	4.6
183	0.5	9.6	0.5	0.5	0.9	4.1	1.8	12.9	1.6	8.5
188	0.1	7.7	0.4	0.3	2.1	3.6	1.4	8.4	3.0	6.9
193	0.3	2.6	0.2	1.6	1.2	2.7	1.0	5.2	3.0	8.2
198	--	1.6	0.2	0.6	0.6	0.3	0.4	2.1	1.0	3.0
203	--	0.5	--	0.2	0.6	0.9	0.1	1.0	--	1.6
208	--	--	--	--	0.3	0.3	0.1	0.5	--	0.3
213	--	--	--	0.1	--	--	--	--	--	--
218	--	--	--	--	--	--	--	--	--	--
Total	37.0	63.0	88.7	11.3	56.8	43.2	28.7	71.3	52.4	47.6
Number of females	278	476	1,071	136	193	146	392	975	160	145

APPENDIX TABLE 4.—Length distributions of ovarian stages by season and cruise—*Continued.*
[UN = undeveloped; D = developing; P = pink; R = ripe]

Total length	Spring									
	Apr. 26-28, 1957		May 29-31, 1957		June 11-22, 1958		Apr. 30-May 3, 1960		Apr. 28-May 1, 1961	
	UN + D	P + R	UN + D	P + R	UN + D	P + R	UN + D	P + R	UN + D	P + R
mm	%	%	%	%	%	%	%	%	%	%
53	--	--	--	--	--	--	--	--	--	--
58	--	--	0.3	--	--	--	--	--	--	--
63	0.2	--	--	--	--	--	--	--	--	--
68	--	--	--	--	--	--	--	--	--	--
73	0.2	--	--	--	--	--	--	--	0.2	--
78	0.3	--	0.5	--	--	--	--	--	0.2	--
83	1.6	--	0.3	--	--	--	0.2	--	0.3	--
88	1.3	--	--	--	--	--	--	--	0.5	--
93	1.8	--	--	--	0.1	--	0.9	--	2.5	--
98	3.8	--	0.3	--	0.2	--	0.4	--	2.4	--
103	4.2	--	0.3	--	0.4	--	2.0	--	3.2	--
108	2.6	--	0.5	--	0.2	--	4.2	--	2.5	--
113	3.0	--	1.1	--	0.9	--	5.3	--	2.5	0.2
118	2.4	--	0.8	--	1.3	--	5.5	0.2	1.5	--
123	3.2	0.2	1.1	--	2.2	--	4.6	--	0.5	--
128	5.9	0.2	1.6	--	2.9	--	2.4	--	0.8	--
133	7.8	0.3	3.2	--	4.6	--	4.0	--	0.5	--
138	6.4	0.5	2.9	--	5.7	0.1	5.7	0.7	2.0	0.2
143	4.2	1.2	3.2	0.8	4.6	0.9	3.1	1.3	1.7	0.4
148	2.2	0.8	1.1	1.6	3.4	1.6	2.9	1.6	1.2	1.2
153	0.6	0.5	1.1	1.1	4.0	2.5	2.4	2.0	1.5	2.7
158	1.1	0.8	0.3	0.5	3.8	2.8	3.1	2.0	1.5	2.6
163	2.2	1.2	1.3	3.5	3.1	3.8	3.3	4.0	1.0	3.5
168	2.2	1.6	1.1	7.2	3.2	3.0	3.5	4.0	1.5	7.2
173	2.2	3.0	1.3	7.5	3.3	3.9	3.5	5.0	2.4	10.4
178	1.9	2.5	1.3	8.8	3.6	4.4	4.4	4.7	1.0	12.5
183	3.4	2.4	1.6	12.7	3.7	5.5	2.2	2.4	1.0	8.5
188	3.7	4.0	1.9	11.3	4.1	4.1	2.2	1.6	1.2	6.7
193	4.2	2.3	1.3	6.7	2.8	3.4	1.5	0.9	0.7	5.1
198	2.6	1.0	--	6.2	1.7	2.6	0.2	1.1	--	2.2
203	0.8	0.5	0.5	2.4	0.4	0.7	0.4	0.2	0.2	1.2
208	0.6	0.2	--	0.8	0.2	0.1	0.2	0.2	--	0.5
213	0.2	--	--	--	--	0.1	--	--	--	0.2
218	--	--	--	--	--	0.1	--	--	--	--
Total	76.8	23.2	28.9	71.1	60.4	39.6	68.1	31.9	34.5	65.5
Number of females	480	144	108	265	1,098	713	310	144	205	384

APPENDIX TABLE 4.—Length distributions of ovarian stages by season and cruise—*Continued.*
[UN = undeveloped; D = developing; P = pink; R = ripe]

Total length	Summer									
	July 17-30, 1957		Aug. 13-20, 1957		Aug. 22-28, 1962		Sept. 25-28, 1962		July 20-22, 1967	
	UN + D	P + R	UN + D	P + R	UN + D	P + R	UN + D	P + R	UN + D	P + R
mm	%	%	%	%	%	%	%	%	%	%
53	--	--	--	--	--	--	--	--	--	--
58	--	--	--	--	--	--	--	--	--	--
63	--	--	--	--	--	--	--	--	--	--
68	--	--	--	--	--	--	--	--	--	--
73	--	--	--	--	--	--	--	--	0.3	--
78	--	--	0.3	--	--	--	0.3	--	--	--
83	0.2	--	0.2	--	0.2	--	0.3	--	--	--
88	0.2	--	0.5	--	--	--	--	--	--	--
93	0.9	--	0.9	--	0.8	--	--	--	0.7	--
98	1.3	--	1.9	--	0.6	--	0.8	--	1.3	--
103	2.2	--	3.3	--	0.6	--	0.8	--	7.4	--
108	2.6	--	3.9	0.1	3.3	--	1.6	--	10.1	--
113	4.0	--	3.8	--	4.8	--	0.8	--	13.4	--
118	4.9	--	3.3	--	3.5	--	0.8	--	13.7	--
123	2.9	--	2.8	--	3.3	--	1.3	--	8.1	--
128	1.5	--	1.3	--	3.5	--	1.1	--	3.7	--
133	4.4	--	3.2	--	2.9	--	0.5	--	3.7	--
138	5.4	0.2	4.4	0.2	1.7	--	1.6	--	5.4	0.3
143	4.8	0.4	4.0	0.2	1.3	--	0.8	--	1.0	0.3
148	3.9	0.4	4.3	0.8	1.7	--	0.8	--	2.4	--
153	2.9	0.8	2.3	0.4	1.0	0.2	2.1	--	3.4	0.7
158	1.1	0.2	3.2	0.5	1.7	0.2	1.3	--	2.4	1.3
163	3.1	0.4	6.2	0.8	6.5	0.4	3.6	1.1	1.3	0.3
168	3.1	2.0	6.0	1.8	9.1	1.0	6.4	1.3	2.7	2.0
173	4.2	4.6	7.2	2.1	10.7	1.5	9.9	1.9	2.7	1.3
178	3.5	3.8	6.5	1.2	14.7	1.9	18.3	1.9	1.0	3.4
183	3.3	5.1	5.5	2.3	10.7	1.0	14.8	2.4	1.0	1.7
188	3.3	7.5	4.3	2.4	6.3	--	9.9	0.8	0.7	1.3
193	2.6	5.0	2.8	1.1	2.5	0.4	6.4	1.3	0.7	--
198	0.9	1.3	1.1	0.9	0.8	--	2.9	0.8	--	0.3
203	0.2	0.9	1.1	0.5	1.0	--	1.1	--	--	--
208	--	--	0.3	0.1	0.2	--	--	--	--	--
213	--	--	--	--	--	--	0.3	--	--	--
218	--	--	--	--	--	--	--	--	--	--
Total	67.4	32.6	84.6	15.4	93.4	6.6	88.5	11.5	87.1	12.9
Number of females	305	149	738	136	487	34	330	43	259	39

APPENDIX TABLE 5.—Length distributions by sex for season and cruise.

Total length	Fall				Winter					
	Nov. 20-25, 1957		Nov. 11-18, 1964		Jan. 18-28, 1960		Jan. 16-Feb. 22, 1962		Feb. 5-7, 1964	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
mm	%	%	%	%	%	%	%	%	%	%
53	--	--	--	--	--	--	--	--	--	--
58	--	--	--	0.2	--	--	--	--	--	--
63	--	--	--	0.2	--	--	--	--	--	--
68	0.2	--	0.1	0.7	--	--	--	--	--	--
73	--	--	--	0.9	--	--	--	0.1	--	0.3
78	0.5	--	0.2	1.1	0.7	0.3	--	0.1	--	0.3
83	0.9	0.5	0.4	1.1	1.7	2.3	--	0.1	0.7	0.7
88	0.2	0.5	0.4	1.1	3.7	3.4	0.1	0.1	2.1	0.7
93	0.9	--	0.2	1.0	3.7	6.7	0.1	0.3	5.2	1.6
98	3.9	0.7	1.7	1.1	3.4	5.1	0.6	0.3	6.2	3.2
103	7.7	2.3	4.9	2.6	2.0	7.6	0.8	0.2	5.9	5.8
108	10.0	5.2	9.3	6.0	2.0	2.5	1.6	0.6	4.5	1.3
113	10.3	5.6	14.6	8.8	1.7	1.4	1.8	0.7	4.5	1.9
118	8.2	5.2	16.5	12.8	3.4	1.1	3.2	0.3	6.6	2.6
123	9.1	3.2	12.1	13.4	7.6	2.0	3.6	1.8	3.1	3.6
128	14.0	2.8	11.3	12.4	20.4	3.1	9.0	1.8	7.9	6.1
133	12.0	2.9	8.6	7.3	18.6	2.5	20.4	1.3	9.3	4.2
138	9.8	3.1	6.2	4.1	15.4	3.9	26.4	0.9	10.0	3.2
143	6.4	4.9	4.2	3.0	9.6	0.8	21.3	0.4	17.6	3.6
148	5.2	3.7	4.2	3.4	2.7	2.5	7.4	0.8	11.0	1.3
153	0.5	4.1	2.6	4.1	2.0	5.1	1.8	1.6	4.1	2.9
158	--	4.5	0.9	2.4	0.5	5.3	0.8	2.8	0.7	3.2
163	--	4.9	1.0	2.4	--	5.6	0.6	6.6	--	2.3
168	--	7.4	0.4	1.4	0.5	8.7	0.1	10.0	--	3.6
173	0.2	8.6	--	2.0	0.2	5.6	0.1	17.0	--	4.2
178	--	6.9	0.1	1.4	0.2	7.6	0.1	16.3	0.3	6.5
183	--	10.1	0.1	1.2	--	4.8	0.1	15.4	0.3	10.0
188	--	8.0	--	0.8	--	5.3	0.1	9.7	--	9.7
193	--	2.8	--	1.8	--	3.7	--	6.3	--	11.4
198	--	1.6	--	0.9	--	1.1	--	2.7	--	3.9
203	--	0.5	--	0.2	--	1.4	--	1.2	--	1.6
208	--	--	--	0.1	--	0.6	--	0.6	--	0.3
213	--	--	--	0.1	--	--	--	--	--	--
218	--	--	--	--	--	--	--	--	--	--
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Number of shrimp	441	755	863	1,233	408	356	881	1,386	290	309

APPENDIX TABLE 5.—Length distributions by sex for season and cruise.—*Continued.*

Total length	Spring									
	Apr. 26-28, 1957		May 29-31, 1957		June 11-22, 1958		Apr. 30-May 3, 1960		Apr. 28-May 1, 1961	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
mm	%	%	%	%	%	%	%	%	%	%
53	--	--	--	--	--	--	--	--	--	--
58	--	--	--	0.3	--	--	--	--	--	--
63	--	0.2	0.5	--	--	0.1	--	--	--	--
68	0.2	--	--	--	--	--	--	--	--	--
73	0.4	0.2	--	--	--	--	--	--	--	0.2
78	0.8	0.3	--	0.5	--	--	--	--	--	0.2
83	1.0	1.6	--	0.3	--	--	--	0.2	0.6	0.3
88	1.8	1.3	0.5	--	0.3	--	--	--	0.8	0.5
93	5.7	1.8	2.1	--	0.3	0.1	0.3	0.9	2.0	2.5
98	5.1	3.8	0.5	0.3	0.4	0.2	0.5	0.4	3.9	2.3
103	5.5	4.2	2.7	0.3	0.7	0.4	2.4	1.9	5.1	3.2
108	5.1	2.6	7.0	0.5	2.5	0.2	4.2	4.1	3.1	2.5
113	6.5	3.0	4.3	1.1	4.2	0.9	4.0	5.4	1.4	2.7
118	8.5	2.4	8.0	0.8	7.6	1.3	7.7	5.6	1.8	1.5
123	12.2	3.4	17.2	1.1	19.1	2.2	16.0	4.5	3.3	0.5
128	7.3	6.1	13.4	1.6	17.6	2.9	19.2	2.6	16.4	0.8
133	8.3	8.2	10.8	3.2	18.3	4.7	18.1	3.9	21.9	0.5
138	9.3	6.9	13.9	3.0	11.2	5.9	13.5	6.3	19.4	2.2
143	10.2	5.5	6.4	4.0	9.1	5.4	7.9	4.3	11.9	2.0
148	8.5	3.0	7.5	2.7	5.5	5.0	1.6	4.3	4.3	2.5
153	2.4	1.1	2.7	2.1	1.7	6.5	1.6	4.5	2.5	4.3
158	0.8	1.9	--	0.8	0.2	6.6	0.8	5.0	0.8	4.0
163	0.2	3.4	--	4.8	0.4	6.9	1.1	7.6	--	5.0
168	--	3.8	--	8.3	--	6.1	0.3	7.7	--	9.0
173	0.2	5.3	0.5	8.8	0.3	7.2	0.5	8.9	0.2	12.7
178	--	4.5	0.5	10.2	0.4	8.1	--	8.9	0.2	13.0
183	--	5.8	0.5	14.2	0.1	9.1	0.3	4.5	0.2	9.7
188	--	7.6	--	13.1	0.1	8.2	--	3.7	--	8.0
193	--	6.3	--	8.0	--	6.2	--	2.4	0.2	5.7
198	--	3.5	0.5	6.2	--	4.3	--	1.3	--	2.2
203	--	1.3	0.5	3.0	--	1.2	--	0.7	--	1.3
208	--	0.8	--	0.8	--	0.2	--	0.4	--	0.5
213	--	0.2	--	--	--	0.1	--	--	--	0.2
218	--	--	--	--	--	--	--	--	--	--
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Number of shrimp	495	624	187	373	1,136	1,811	379	463	489	601

APPENDIX TABLE 5.—Length distributions by sex for season and cruise.—*Continued.*

Total length	Summer									
	July 17-30, 1957		Aug. 13-20, 1957		Aug. 22-28, 1962		Sept. 25-28, 1962		July 20-22, 1967	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
mm	%	%	%	%	%	%	%	%	%	%
53	--	--	--	--	--	--	--	--	--	--
58	--	--	--	--	--	--	--	--	--	--
63	--	--	--	--	--	0.2	--	--	--	--
68	--	--	--	--	--	--	--	--	--	--
73	--	--	--	--	0.3	--	--	--	--	0.3
78	--	--	0.4	0.3	--	--	--	0.3	--	--
83	--	0.2	0.4	0.2	--	0.2	--	0.3	0.4	--
88	1.7	0.2	1.1	0.5	0.5	--	0.3	--	--	--
93	2.3	0.9	1.3	0.9	1.5	0.8	0.5	--	0.8	0.7
98	3.4	1.3	2.3	1.9	3.3	0.6	0.8	0.8	3.2	1.3
103	5.7	2.2	4.5	3.3	6.9	0.6	1.9	0.8	11.2	7.4
108	5.1	2.6	5.9	4.0	8.1	3.2	0.8	1.6	9.2	10.0
113	8.3	4.0	4.1	3.8	7.9	4.8	3.0	0.8	13.8	13.4
118	7.1	4.8	7.4	3.3	3.8	3.4	2.7	0.8	10.0	13.6
123	9.4	2.9	14.9	2.8	8.1	3.2	3.2	1.3	12.3	8.0
128	11.4	1.5	13.0	1.3	12.0	2.9	5.4	1.3	10.0	3.7
133	12.6	4.4	15.4	3.2	19.8	2.9	16.4	0.5	11.5	3.7
138	13.8	5.5	15.4	4.6	18.6	1.7	28.3	1.9	6.8	5.7
143	13.4	5.0	8.9	4.2	4.3	1.3	25.5	0.8	7.2	1.3
148	2.6	4.4	4.4	5.2	4.1	1.7	8.8	0.8	0.8	2.7
153	1.7	3.7	0.6	2.8	0.5	1.1	1.3	2.1	1.6	4.0
158	0.9	1.5	--	3.8	--	1.9	0.5	1.3	0.8	3.7
163	--	3.5	--	7.0	0.3	6.8	--	4.5	0.4	1.7
168	0.3	5.0	--	7.8	--	10.1	0.3	7.7	--	4.7
173	--	8.8	--	9.1	--	12.0	0.3	11.7	--	4.0
178	0.3	7.2	--	7.6	--	17.6	--	19.9	--	4.4
183	--	8.4	--	7.8	--	11.8	--	17.1	--	2.7
188	--	10.8	--	6.7	--	6.3	--	10.9	--	2.0
193	--	7.9	--	4.0	--	2.7	--	7.7	--	0.7
198	--	2.2	--	1.9	--	1.0	--	3.7	--	0.3
203	--	1.1	--	1.5	--	1.0	--	1.1	--	--
208	--	--	--	0.5	--	0.2	--	--	--	--
213	--	--	--	--	--	--	--	0.3	--	--
218	--	--	--	--	--	--	--	--	--	--
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Number of shrimp	350	456	798	874	393	525	373	376	251	299

APPENDIX TABLE 6.—Length distributions by cruise and sex.

Total length	Apr. 26-28, 1957		May 29-31, 1957		July 17-30, 1957		Aug. 13-20, 1957		Nov. 20-25, 1957	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
mm	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
53	--	--	--	--	--	--	--	--	--	--
58	--	--	--	1	--	--	--	--	--	--
63	--	1	1	--	--	--	--	--	--	--
68	1	--	--	--	--	--	--	--	1	--
73	2	1	--	--	--	--	--	--	--	--
78	4	2	--	2	--	--	3	3	2	--
83	5	10	--	1	--	1	3	2	4	4
88	9	8	1	--	6	1	9	4	1	4
93	28	11	4	--	8	4	10	8	4	--
98	25	24	1	1	12	6	18	17	17	5
103	27	26	5	1	20	10	36	29	34	17
108	25	16	13	2	18	12	47	35	44	39
113	32	19	8	4	29	18	33	33	46	42
118	42	15	15	3	25	22	59	29	36	39
123	61	21	32	4	33	13	119	24	40	24
128	36	38	25	6	40	7	104	11	62	21
133	41	51	20	12	44	20	123	28	53	22
138	46	43	26	11	48	25	123	40	43	23
143	51	34	12	15	47	23	71	37	28	37
148	42	19	14	10	9	20	35	45	23	28
153	12	7	5	8	6	17	5	24	2	31
158	4	12	--	3	3	7	--	33	--	34
163	1	21	--	18	--	16	--	61	--	37
168	--	24	--	31	1	23	--	68	--	56
173	1	33	1	33	--	40	--	80	1	65
178	--	28	1	38	1	33	--	67	--	52
183	--	36	1	53	--	38	--	68	--	77
188	--	48	--	49	--	49	--	59	--	61
193	--	40	--	30	--	36	--	35	--	21
198	--	22	1	23	--	10	--	17	--	12
203	--	8	1	11	--	5	--	13	--	4
208	--	5	--	3	--	--	--	4	--	--
213	--	1	--	--	--	--	--	--	--	--
218	--	--	--	--	--	--	--	--	--	--
Total	495	624	187	373	350	456	798	874	441	755
Number of stations	7		3		9		18		14	

APPENDIX TABLE 6.—Length distributions by cruise and sex.—*Continued.*

Total length	Aug. 22-28, 1962		Sept. 25-28, 1962		Feb. 5-7, 1964		Nov. 11-18, 1964		July 20-22, 1967	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
mm	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
53	--	--	--	--	--	--	--	--	--	--
58	--	--	--	--	--	--	--	2	--	--
63	--	1	--	--	--	--	--	2	--	--
68	--	--	--	--	--	--	1	8	--	--
73	1	--	--	--	--	1	--	11	--	1
78	--	--	--	1	--	1	2	13	--	--
83	--	1	--	1	2	2	3	14	1	--
88	2	--	1	--	6	2	3	13	--	--
93	6	4	2	--	15	5	2	12	2	2
98	13	3	3	3	18	10	15	14	8	4
103	27	3	7	3	17	18	42	32	28	22
108	32	17	3	6	13	4	80	74	23	30
113	31	25	11	3	13	6	127	110	35	40
118	15	18	10	3	19	8	143	159	25	41
123	32	17	12	5	9	11	105	167	31	24
128	47	15	20	5	23	19	97	154	25	11
133	78	15	61	2	27	13	74	90	29	11
138	73	9	100	7	29	10	53	50	17	17
143	17	7	95	3	51	11	36	37	18	4
148	16	9	33	3	32	4	36	42	2	8
153	2	6	5	8	12	9	22	50	4	12
158	--	10	2	5	2	10	8	29	2	11
163	1	36	--	17	--	7	9	29	1	5
168	--	53	1	29	--	11	3	17	--	14
173	--	63	1	44	--	13	--	25	--	12
178	--	93	--	75	1	20	1	17	--	13
183	--	62	--	64	1	31	1	15	--	8
188	--	33	--	41	--	30	--	10	--	6
193	--	14	--	29	--	35	--	22	--	2
198	--	5	--	14	--	12	--	11	--	1
203	--	5	--	4	--	5	--	2	--	--
208	--	1	--	--	--	1	--	1	--	--
213	--	--	--	1	--	--	--	1	--	--
218	--	--	--	--	--	--	--	--	--	--
Total	393	525	373	376	290	309	863	1,233	251	299
Number of stations	10		8		6		21		6	

APPENDIX TABLE 6.—Length distributions by cruise and sex.—*Continued.*

Total length	June 11-22, 1958		Jan. 18-28, 1960		Apr. 30-May 3, 1960		Apr. 28-May 1, 1961		Jan. 16-Feb. 22, 1962	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
mm	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
53	--	--	--	--	--	--	--	--	--	--
58	--	--	--	--	--	--	--	--	--	--
63	--	1	--	--	--	--	--	--	--	--
68	--	--	--	--	--	--	--	--	--	--
73	--	--	--	--	--	--	--	1	--	1
78	--	--	3	1	--	--	--	1	--	1
83	--	--	7	8	--	1	3	2	--	2
88	3	--	15	12	--	--	4	3	1	2
93	3	2	15	24	1	4	10	15	1	4
98	4	3	14	18	2	2	19	14	5	4
103	8	7	8	27	9	9	25	19	7	3
108	28	3	8	9	16	19	15	15	14	8
113	48	17	7	5	15	25	7	16	16	10
118	86	23	14	4	29	26	9	9	28	4
123	218	40	31	7	61	21	16	3	32	25
128	201	53	83	11	73	12	80	5	79	25
133	209	85	76	9	69	18	107	3	180	18
138	128	107	63	14	51	29	95	13	232	13
143	104	98	39	3	30	20	58	12	188	5
148	62	91	11	9	6	20	21	15	65	11
153	19	117	8	18	6	21	12	26	16	22
158	2	119	2	19	3	23	4	24	7	39
163	4	125	--	20	4	35	--	30	5	91
168	--	111	2	31	1	36	--	54	1	138
173	3	130	1	20	2	41	1	77	1	236
178	4	146	1	27	--	41	1	79	1	225
183	1	166	--	17	1	21	1	58	1	213
188	1	149	--	19	--	17	--	48	1	135
193	--	113	--	13	--	11	1	34	--	88
198	--	78	--	4	--	6	--	13	--	38
203	--	22	--	5	--	3	--	8	--	17
208	--	4	--	2	--	2	--	3	--	8
213	--	1	--	--	--	--	--	1	--	--
218	--	--	--	--	--	--	--	--	--	--
Total	1,136	1,811	408	356	379	463	489	601	881	1,386
Number of stations	28		13		10		12		24	

SEX PHEROMONE ACTIVITY OF THE MOLTING HORMONE, CRUSTECDYSONE, ON MALE CRABS

(*Pachygrapsus crassipes*, *Cancer antennarius*, AND *C. anthonyi*)

JAMES S. KITTREDGE,[1] MICHELLE TERRY,[1] AND FRANCIS T. TAKAHASHI[2]

ABSTRACT

The pheromone released by premolt female *Pachygrapsus crassipes* is a heat stable non-ionic polar lipid. The coincidence of the release of the pheromone and the nubial molt suggested that the molting hormone, crustecdysone, may also function as a sex pheromone. Adult male crabs were observed to display typical precopulatory behavior when exposed to dilute solutions of crustecdysone. The threshold concentration for behavioral response was found to be 10^{-13} M for *P. crassipes*, 10^{-10} M for *Cancer antennarius* and 10^{-8} M for *C. anthonyi*. These findings provide the basis for a theory of the evolution of pheromone communication in the Arthropoda.

The dominant position of chemoreception in the behavior of marine invertebrates and the implication of sex pheromones in the reproductive activities of many species is supported by many behavioral observations, but no pheromone has yet been characterized from the marine environment. In many marine decapod Crustacea copulation takes place immediately after the female molts. The male of the species recognizes the premolt condition of the female, is attracted to her, and usually seizes and carries her until she molts. This recognition at a distance has been reported for many genera of Crustacea (Hay, 1905; L. Agassiz in Verrill, 1908; Needler, 1931; Burkenroad, 1947; Williamson, 1953; Hughes and Matthiessen, 1962; Knudsen, 1964; Snow and Neilsen, 1966). Ryan (1966) described the search and display behavior exhibited by male *Portunus anguinotentus* when a premolt female crab was placed in the holding tank with them. Each male became active, walked about on the tips of its dactyls, elevated its body, and extended its chelae. When thus stimulated they often attempted to pull any crab they met into a precopulatory carrying position. Ryan demonstrated that this behavior is released by a sex pheromone in the urine of the premolt female crab.

METHODS

BIOASSAY

Observation vessels for determining the response of male *Pachygrapsus crassipes* to dilute solutions of molting hormone were constructed from 4-liter beakers. With a glass blowing torch and the edge of a carbon flat we formed an indent in the side of each beaker approximately 4 cm deep, parallel to and 3 cm above the bottom of the beaker. The outside of the beakers was masked with black paint with the exception of an 8 cm window opposite the indent. When a crab was placed in seawater in the observation chamber, they always scurried into the niche between the bottom of the beaker and the indent. If the seawater contained molting hormone, the crabs were stimulated to come out of the niche and assume a premating stance. The time elapsing after adding a solution of crustecdysone in seawater to an empty vessel containing a male crab until the crab elevated its cephalothorax in a typical stance was noted. Six crabs were timed at each concentration of

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crustecdysone, and fresh male crabs were used for each different concentration. The male crabs were held for several days in isolation from female crabs before testing.

ISOLATION COLUMNS

Columns (5 × 50 cm) of Amberlite XAD-2, a divinylbenzene polymer, were found to be effective in the recovery of polar steroids from seawater. The columns were washed with three void volumes of water to remove the salts, and the polar steroids were eluted with three volumes of 60% ethanol. The more nonpolar lipids were removed with 95% ethanol. After repeated use the columns were reconditioned by cycling through 95% ethanol, diethyl ether, hexane, diethyl ether, ethanol, and water.

FRACTIONATION COLUMNS

Chromosorb 102, which is an 80/100 mesh fraction of Amberlite XAD-2, in a 0.9 × 100 cm column was used to fractionate the polar lipids. This column was eluted with a gradient of ethanol (20% to 80%) (Hori, 1969). The gradient was formed by an Isco Dialagrad dual pump[3] with the following settings: 40 ml/hr; reservoir for "A" pump, 80% ethanol; reservoir for "B" pump, 20% ethanol; percentage settings for "B" pump, 100, 100, 90, 80, 70, 60, 50, 30, 15, 0, 0 (this pump makes five intermediate linear steps between each setting); total time 16 hr. The fractionation was monitored at 254 μ with an Isco Model UA-2 UV monitor and fractions were collected in an Isco Model 327 fraction collector.

A silicic acid column (0.6 × 30 cm) eluted with chloroform-ethanol (5:1, v/v) and monitored in the UV was employed for further fractionation (Horn et al., 1968).

OBSERVATIONS ON THE PREMATING BEHAVIOR AND THE SEX PHEROMONE OF *Pachygrapsus crassipes*

Although the premating behavior of the lined shore crab, *Pachygrapsus crassipes,* as described by others (Hiatt, 1948; Bovbjerg, 1960), did not include the typical stance of other Brachyura, the abundance and ease of collection of this species prompted us to re-examine their behavior. We found that male *P. crassipes,* in the presence of a premolt female, exhibit an easily recognizable stance. The males elevate their cephalothoraxes and tilt the anterior margin up. They walk on the tips of the dactyls of their first three pairs of walking legs and extend their fourth pair horizontally backwards. The chelipeds are partially extended but lowered, as opposed to the elevated defensive position. When thus stimulated they will often attempt to seize any other *P. crassipes* they encounter, male or female, and turn them over into the holding position with which they maintain control of a premolt female. This behavior compares with that described by Ryan (1966) for male *P. sanguinolentus.* There were two additional characteristics of the male *P. crassipes* behavior that paralleled the premating behavior of *Cancer magister* as described by Snow and Neilsen (1966). They observed that the male *C. magister,* while carrying the female, frequently extended his fourth pair of walking legs straight back. We have observed that *C. magister* will thus extend his legs while holding his body elevated when stimulated by the sex pheromone before he seizes the female, as does *P. crassipes.* Snow and Neilsen (1966) also noted that "on occasion the male would rise up on the tips of his walking legs and raise the female up into an elevated position nearly 6 inches off the bottom of the tank. This movement would be accompanied by a continuous flexing of the male's abdominal flap." A frequent observation with pairs of *P. crassipes* was that they would stand facing each other, both with their legs extended and body elevated, but with the male higher. In this position the female would lower her abdominal flap slowly and then flex it rapidly but not into a completely retracted position. She would repeat this movement several times. The male would then repeat an identical movement of his abdominal flap.

[3] Reference to commercial products does not imply endorsement.

This aspect of their behavior could be interpreted as a courting gesture, but in the context of chemical communication we prefer to interpret this as a fanning motion facilitating the distribution of pheromones that may be aphrodisiac in nature. Commercial fishermen for both the American lobster, *Homarus americanus*, and the California spiny lobster, *Panulirus interruptus*, have suspected that the males of each species could be used to attract the females.

A single active premolt female *Pachygrapsus crassipes* released sufficient pheromone to stimulate all of the males in a 25-gal recirculating aquarium. A single female *P. crassipes* in an aerated 2-gal container released a pheromone which stimulated a male *Cancer antennarius* to exhibit a premating stance.

With these observations providing the bioassay, we examined the nature of the sex pheromone. Water which had contained a premolt female crab was boiled for 10 min, cooled, and aerated. The active principle was still present. The active principle was not retained by cation nor anion exchange resins nor by charcoal. The active substance could, however, be extracted from "active seawater" with isopropanol/diethyl ether. These observations, together with the premolt condition of the active females, led us to suspect that the females might be releasing molting hormone into the water, and that this steroid might be functioning as a sex pheromone.

BIOASSAY OF CRUSTECDYSONE

We soon confirmed that dilute solutions of crustecdysone (β-ecdysone, 20R-hydroxyecdysone, ecdysterone, isoinokosterone), which is one of the molting hormones of Crustacea (Horn et al., 1968), elicited a typical response from male *P. crassipes*. In order to establish the threshold concentration that would release response we standardized the conditions for the bioassay. As described under "Methods," the test adopted allowed the male crab to be flooded with a known concentration of the steroid in seawater, in contrast to the diffusion techniques often employed. *P. crassipes* proves to be ideal for this mode of testing because they are an intertidal species and in nature normally leave the tide pools at low tide to feed on the rocks. They were not disturbed on being placed in a wet empty observation vessel and sought out the artificial niche provided. On flooding with seawater they would remain in the niche for long periods or occasionally come out to explore briefly and then return to the niche. When a male *P. crassipes* was flooded with a solution of crustecdysone in seawater, he was stimulated to come out of the niche and explore the vessel and then to assume the premating stance. The time elapsing until the crab raised its body to assume the stance was found to be a function of the concentration of crustecdysone. Although the male crabs would often exhibit a full stance in a brightly illuminated laboratory at the higher concentrations of crustecdysone, the response was often erratic. All of the bioassays were conducted in an isolated room illuminated with an Eastman darkroom lamp with a 15-w bulb and an Eastman No. 00 yellow filter. The observer was stationed quietly before the "window" of the test vessel. On removal from the test vessel the male crabs were transferred to a 2-liter beaker of seawater to rinse off the extraneous crustecdysone and then were transferred to a small aquarium for further observation. In spite of the handling during the two transfers, male crabs that had been stimulated to display a premating stance in the observation vessel usually resumed this posture shortly after being transferred to the aquarium. When thus stimulated they often attempted to seize any other male crab in the aquarium.

All of the male *P. crassipes* utilized in establishing the response curve were collected at the same time and in the same area where we had just succeeded in collecting a number of premolt females. They were all held for three or more days in isolation from any female crabs. The curve was started with a concentration of 10^{-5} M crustecdysone in filtered seawater. At this concentration the response was rapid, averaging 7 sec. Succeeding tests were performed with ten-fold dilutions of the crustecdysone. Fresh solutions of crustecdysone were prepared after three steps of dilution or at the start of each day's testing. Previous experience had

demonstrated that dilute solutions in seawater lost some or all of their activity on storage even at 0° C, presumably through bacterial degradation or adsorption. It was planned that six male crabs would be tested at each concentration and that the five most consistent times would be averaged; however, the response was found to be remarkably uniform and in all but three cases all six crabs responded within a narrow time range. There was no sharp threshold of concentration. The average response times plotted as a smooth curve extending to 10^{-3} M crustecdysone concentration where the average response time was 22 min (Figure 1). No response was observed at 10^{-14} M. The scatter of response times was greatest at 10^{-5} M, probably because of the short-term disturbance of the male crab during flooding. The standard deviation of the normalized response times for 45 crabs, from 10^{-6} M to 10^{-13} M crustecdysone, was 8.6.

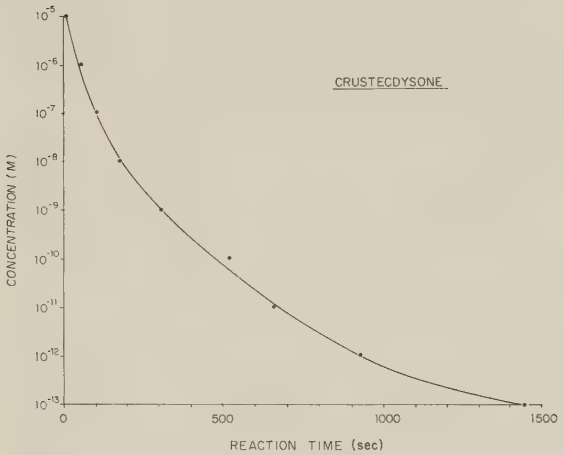

FIGURE 1.—Time elapsed following immersion of male *Pachygrapsus crassipes* in seawater solutions of crustecdysone before the body elevation phase of the precopulatory behavior.

Since the precopulatory behavior of males in the presence of premolt females appears to be general among the Brachyura, we examined the response of two species of *Cancer* to crustecdysone. Both *C. antennarius* and *C. anthonyi* displayed typical premating behavior when exposed to dilute solutions of crustecdysone. When the response time vs. concentration study was carried out with these two species each yielded a response curve similar to that developed by *P. crassipes*. There was, however, a marked difference. There was an abrupt break in the response yielding a distinct threshold at 10^{-10} M for *C. antennarius* and 10^{-8} M for *C. anthonyi*.

We then attempted to determine if *C. antennarius* males could detect a gradient in the concentration of crustecdysone. For this purpose we employed a simple "T" maze with a baffle at the head of each arm separating the seawater sources and forming mixing chambers. Five male *C. antennarius* were placed at the head of the maze and the water flows from each arm balanced. While seawater alone was flowing through the maze the *C. antennarius* remained quiet in the two corners at the origin of the maze. When a flow of crustecdysone solution was added to the mixing chamber at the head of one arm all five of the male *C. antennarius* soon became active. They explored up and down each arm of the maze but demonstrated no tendency to select the arm containing the crustecdysone. While these tests did not indicate any ability to detect or follow up a gradient, there was a positive response to the crustecdysone. All five of the male crabs were stimulated simultaneously to undertake an active exploratory behavior when the crustecdysone was introduced.

DEVELOPMENT OF THE TECHNIQUES FOR THE ISOLATION AND FRACTIONATION OF THE SEX PHEROMONE(S)

Liquid-liquid extraction procedures are inefficient for the recovery of trace quantities of polar lipids. Columns of Amberlite XAD-2 have been employed for the recovery of steroids from urine (Bradlow, 1968; Shackleton, Sjövall, and Wisén, 1970). Recently, Hori (1969) has employed a column of this resin eluted with a li-

near gradient of ethanol in water for the fractionation of the phytoecdysones. We have found that a column of XAD-2 could be used to recover traces of crustecdysone from seawater and from crab urine. Using the above two columns we have examined the seawater in which female *P. crassipes, C. magister,* and *C. productus* had been maintained for 3 to 6 hr. The product of individual *Cancer* were assayed, while the seawater from two or more *P. crassipes* was combined before extraction. We have also examined the urine of female *C. magister.* The *Cancer* were staged according to Drach (1939), and the female *P. crassipes* were selected for activity by observing the behavior of males in their presence. During our observations we found the female *C. magister* continued to release a pheromone for up to 2 weeks post molt.

The material recovered from the isolation column in 60% ethanol was reduced in volume to a few microliters and transferred in 20% ethanol to the Chromosorb 102 column. Elution of this column yielded an ultraviolet-absorbing peak near the front and two or more succeeding peaks. Each of three stage "D" *C. magister* and one stage "D" *C. productus* studied were found to have released an ultraviolet-absorbing compound that eluted from the column at the same ethanol concentration that a crustecdysone standard did. Both extracts of *P. crassipes* seawater yielded a peak in the position of crustecdysone. One stage "A" *C. magister* also yielded a peak in this position. A stage "C-4" *C. magister* did not yield any peak in the position of crustecdysone nor did urine from a stage "C-4" *C. magister* yield a peak in this position. Also one of the *C. magister* females that had yielded material eluting as crustecdysone while in stage "D" did not yield this substance during molting (stage "E"). The ultraviolet-absorbing fractions corresponding to crustecdysone from the above columns did not have an ultraviolet-absorption spectrum corresponding to that of crustecdysone. The absorption peak included the spectrum of crustecdysone, but had a double peak at a lower wave length. These fractions were concentrated and applied to a silicic acid column. The elution of this column with chloroform-ethanol yielded an ultraviolet-absorbing peak near the front and a peak eluting in the same volume as a crustecdysone standard. The material from this column has an ultraviolet-absorption spectrum that corresponds closely to that of crustecdysone.

We are now developing a derivitization technique that will permit us to subject our final samples to gas chromatography-mass spectrophotometry for structural conformation. Katz and Lensky (1970) have published a technique for the silylation of α-ecdysone for GLC analysis. We have employed silylation techniques with crustecdysone and observed decomposition during GLC.

RESULTS AND CONCLUSIONS

The pheromone released by *P. crassipes* stimulates premating behavior in *C. antennarius.* Male *C. magister* are excited into seizing and clasping female *C. productus* by their pheromone. Crustecdysone mimics the pheromone in its effects on male *P. crassipes, C. antennarius,* and *C. anthonyi* in the release of the premating stance. After exposure to crustecdysone all these species of male crabs attempt to seize other crabs, male or female, and pull them into a precopulatory position. In addition crustecdysone triggers a search behavior in male *C. antennarius.* These observations demonstrate a lack of specificity in the sex pheromones of these species and either that crustecdysone is the sex pheromone or sufficiently similar in molecular structure to the natural pheromones to mimic them.

A possible explanation of the discrepency between our results and those of Ryan (1966) that indicated a species specificity for the sex pheromones in the three species of crabs that he studied may be that some species may respond to deoxycrustecdysone, callinecdysone A (inokosterone) or callinecdysone B (makisterone), other ecdysones that have been isolated from Crustacea, (Gailbraith et al., 1968; Faux et al., 1969), or they may respond to one of the metabolic products of crustecdysone detected in insects (Gailbraith et al., 1969; Moriyama et al.,

1970; Cherbas and Cherbas, 1970; Heinrich and Hoffmeister, 1970).

The isolation and analysis of the material released into seawater by active female *P. crassipes, C. magister,* and *C. productus* demonstrated that a compound could be detected that is eluted from two different columns in the same position as crustecdysone and has a UV absorption spectra that is similar to that of crustecdysone.

The semilog plot of the response times for male *P. crassipes* to varying concentrations of crustecdysone is approximately parabolic, and the scatter of response times at each concentration is remarkably narrow. This, the range of response times, and the continued response of the male crabs after removal from the stimulus, permit an interpretation of the chemoreception of pheromones from dilute solutions. The observations suggest that the pheromone has a high affinity for the receptor site resulting in a long half life for the receptor-pheromone complex. Indeed, one might have postulated that even a polar steroid might be strongly bound to a lipoid receptor in an aqueous medium. It is apparent that the crabs are capable of summating the chemical information for a considerable period of time before a threshold which releases response behavior is reached. Though summation may take place at any level in the nervous system, the simplest interpretation suggests that this takes place at the receptors. This summation of "information quanta" can function either in extremely dilute solutions or, in nature, it would permit the accumulation of subthreshold amounts presented in random turbulences of the current from the source.

This finding has significance in a consideration of the evolution of pheromone communication. It has been suggested that chemical signals between cells were evolved before the evolution of the metazoans and that these signals were later internalized as hormones and synaptic transmitters (Haldane, 1955; Wilson, 1968). In the present instance we have a reversal of this internalization. The Crustacea, having evolved polar steroid hormones to regulate molting, on externalization of the receptor site onto chemoreceptor organs and on alteration of the resorption process in the antennual gland during the premolt stage of the females were then capable of signaling the approach of the nubial molt. This interpretation obviates the concern over the improbability of the simultaneous *de novo* origin of both the genetic information directing the biosynthesis of the pheromone and that concerned with the architecture of the receptor site. We may assume that an unmasking of that portion of the chromosome that specifies the receptor site for the hormone on the membranes of the target organs occurred in the chemosensory neurons. A masking of the active transport system for the hormone from the fluid of the antennule gland of the female is also assumed. These two innovations are reasonably small evolutionary steps and conceptually preferable to the two *de nova* origins that must be assumed otherwise. This evolutionary step, the pheromone function of a hormone, may have been the origin of pheromone communication in the Arthropoda, for once fixed because of its reproductive value, it was then susceptible to a gradual evolutionary drift toward a variety of more specific pheromones.

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CHARACTERISTICS OF SEA-SURFACE TEMPERATURE ANOMALIES

L. E. Eber[1]

ABSTRACT

Sea-surface temperature anomalies in the North Pacific Ocean, constructed from a 14-year series (1949-62) of monthly mean charts, exhibit numerous instances of quasi-stationary behavior. Selected examples from this series reveal a recurring pattern in which the principal feature is a positive or negative cell in the anomaly field, located approximately between lat 30° N and 50° N. The cell in this pattern is partially encircled by anomalies of opposite sign to the north, east, and south in a zone contiguous with the North American coast. This anomaly configuration, viewed with consideration of the associated sea-temperature field, suggests the existence of a standing wave in the current structure. Such a wave could affect the partitioning of the West Wind Drift Current as it approaches the coast and splits into northward and southward flowing branches. Physical data for verification of a standing wave are not available, but dimensional attributes inferred from the sea-temperature anomaly structure conform loosely to theoretical constraints.

The temperatures in the upper mixed layer of the ocean undergo annual cycles, induced by seasonal heating and cooling, which vary from year to year. This variation can be expressed in terms of departures from the mean annual temperature cycle obtained by averaging over a number of years. The magnitudes, areal distributions, and time changes of such departures define anomalous conditions in the surface layer of the sea.

The 14-year series, 1949-62, of monthly sea-surface temperature charts, published by Eber, Saur, and Sette (1968), provides a base for studying temperature variations in the North Pacific Ocean. Monthly anomaly charts were constructed from this series by taking the difference between the sea-surface temperature fields for each month and year and the corresponding monthly normal fields. The latter were obtained by computing the 14-year averages, by month, at grid points. A number of selected examples are presented here to show the character of some of the prominent and long-lasting anomalies that occurred in the North Pacific Ocean between 1949 and 1962.

Many of the features to be discussed are in the vicinity of the transition zone between the subarctic and subtropic oceanographic regions as described by Tully (1964). Through this zone, which is located approximately at lat 35° N to 45° N between long 160° E and 140° W, the surface current flows eastward as the West Wind Drift. The mean surface temperature distribution in this region is essentially zonal with isotherms oriented along the circles of latitude. The chart of the 14-year average for October (Figure 1) illustrates these characteristics. As the West Wind Drift approaches

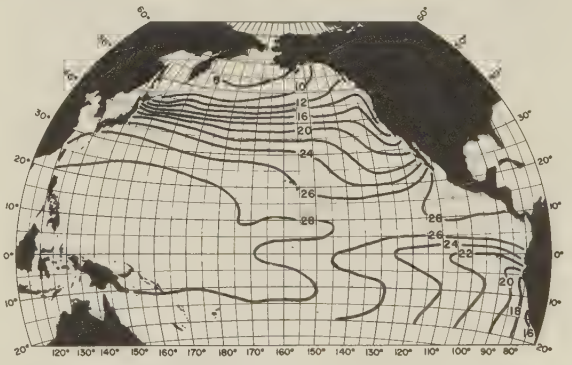

FIGURE 1.—Average sea-surface temperatures of the North Pacific Ocean in October, based on data from the 14-year period 1949-62.

the North American coast, it splits. One part turns north and moves in a counterclockwise trajectory around the Alaska Gyre and the other part turns south to become the California

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Current. The sea-surface isotherms, correspondingly, bend north and south, effecting a much-reduced temperature gradient parallel to the coast.

BEHAVIOR OF SEA-SURFACE TEMPERATURE ANOMALIES

To facilitate description, I shall designate as "warm" or "cold" cells, areas where the magnitude of departure from normal was 1° C or greater. The evolution of an anomaly can be readily followed by noting the configuration of its principal cell, or cells, in successive months. This is evident in the figures used to illustrate selected examples (Figures 2-30). These show

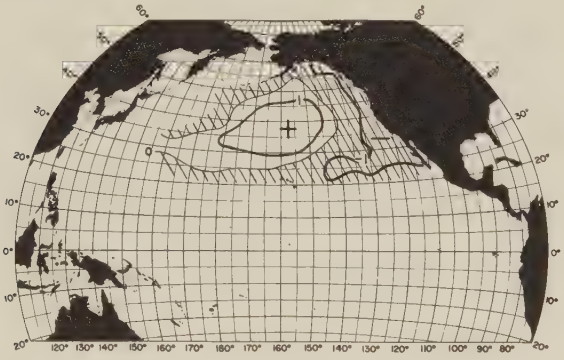

FIGURE 2.—Sea-surface temperature anomaly for January 1949. Hatched areas colder than normal. Heavy lines represent the 1° C anomaly contours which define warm (+) or cold (—) cells.

the sizes and locations of the principal cells, enclosed by heavy lines representing a magnitude of 1° C, in the regions relevant to the discussion. The cells are marked by plus or minus symbols according to the sign of the anomalies. The thin lines represent the zero anomaly contour separating areas of below normal temperature (hatched areas) from those where the temperature was above normal.

A good example of persistence was the warm anomaly present in the eastern North Pacific throughout most of 1949. In January the warm cell (defined by the 1° C anomaly contour) covered most of the area from lat 30° N to 45° N and long 145° W to 180° W. Maximum intensity of the positive departures from normal within this cell exceeded 3° C. Off the North American coast, the temperature anomalies were negative, with magnitudes greater than 1° C in a broad zone from Alaska to the tip of Baja California.

Figures 3-5 show subsequent positions of the warm cell in March, July, and October, respectively. The maximum intensities waxed and waned over this time period, dropping in March, increasing again to more than 3° C in July and diminishing once more in October. Except for a slight northward shift, the warm cell remained essentially stationary. During most of the period, negative anomalies prevailed in the coastal

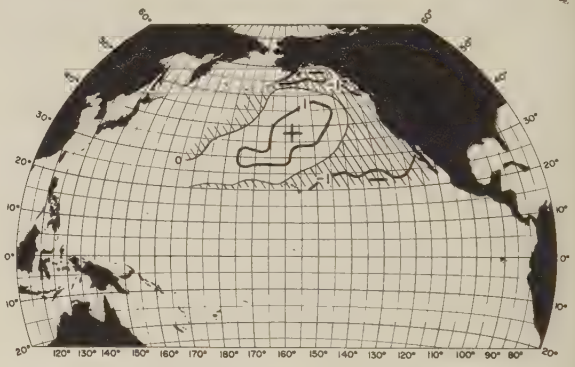

FIGURE 3.—Sea-surface temperature anomaly for March 1949. Hatched areas colder than normal. Heavy lines represent the 1° C anomaly contours which define warm (+) or cold (—) cells.

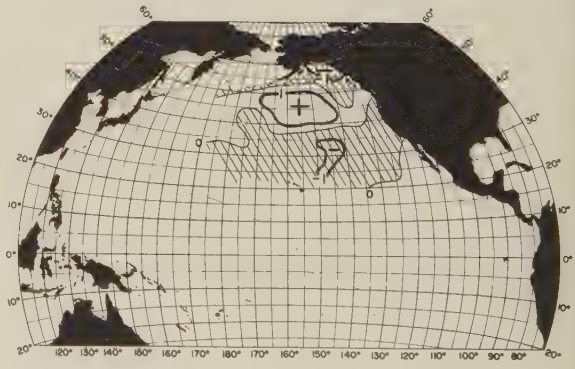

FIGURE 4.—Sea-surface temperature anomaly for July 1949. Hatched areas colder than normal. Heavy lines represent the 1° C anomaly contours which define warm (+) or cold (—) cells.

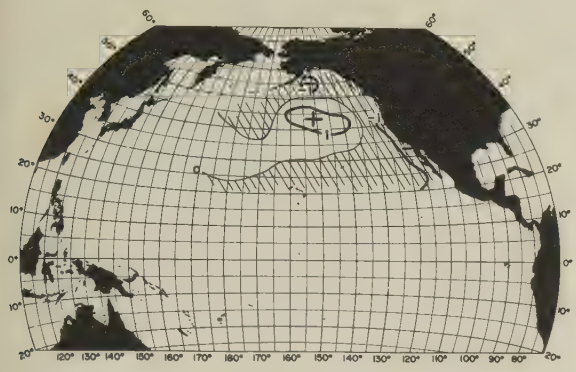

FIGURE 5.—Sea-surface temperature anomaly for October 1949. Hatched areas colder than normal. Heavy lines represent the 1° C anomaly contours which define warm (+) or cold (—) cells.

zone and in a southwestward tongue below 30° N but with fluctuating magnitudes.

The warm cell shrank in November and lost its specific identity in December. However, the general configuration of the colder than normal coastal zone and warmer than normal offshore region persisted through the winter and spring of 1950. In May 1950, the positive anomalies intensified and spread westward to Japan. The pattern stabilized in June and July marked by prominent warm cells in the east and far west portions of the positive area (Figure 6). At the same time, a cold cell in the Gulf of Alaska had begun to grow and push southward; this

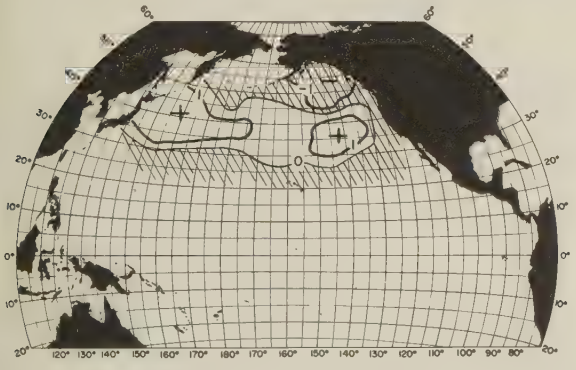

FIGURE 6.—Sea-surface temperature anomaly for July 1950. Hatched areas colder than normal. Heavy lines represent the 1° C anomaly contours which define warm (+) or cold (—) cells.

movement continued through September (Figure 7) accompanied by an eastward expansion of the warm belt. Ultimately, there was a deformation of the broad-scale pattern which had for many months dominated the central and eastern North Pacific, as negative anomalies spread through the Gulf of Alaska.

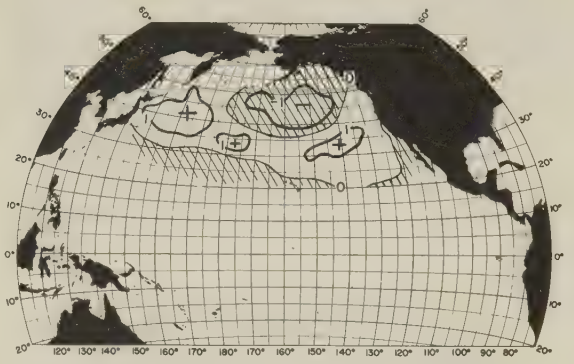

FIGURE 7.—Sea-surface temperature anomaly for September 1950. Hatched areas colder than normal. Heavy lines represent the 1° C anomaly contours which define warm (+) or cold (—) cells.

The years 1951-54 presented no striking examples of persistent sea temperature anomalies. Some relatively large-scale anomalies did develop in this period but they were short-lived. Much of the time the anomaly field was flat and featureless. Midway through 1955, however, a long-term progression of events began to evolve with the development of an anomaly pattern which was characterized by a positive belt that stretched eastward from Japan and a negative zone along most of the North American coast. The anomaly field intensified in August (Figure 8) when maxima exceeded 2° C in the warm cell and an extensive cold area was present in the coastal zone with negative departures exceeding 1° C in magnitude. The negative area stretched southwestward below lat 30° N. This pattern prevailed through November 1955 (Figure 9) but became weaker in December. In January 1956, the positive area contracted to the west while the region of negative anomalies to the east became flat and disorganized. Figure 10 shows the situation in February 1956. The

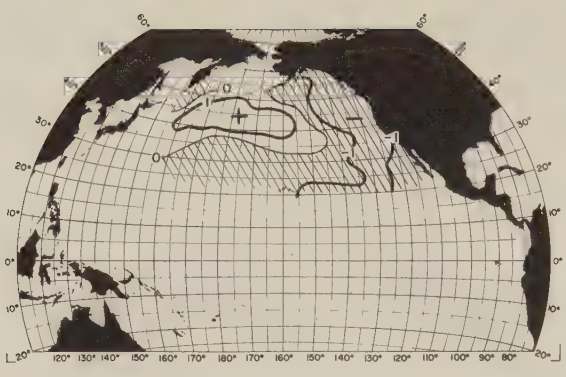

FIGURE 8.—Sea-surface temperature anomaly for August 1955. Hatched areas colder than normal. Heavy lines represent the 1° C anomaly contours which define warm (+) or cold (−) cells.

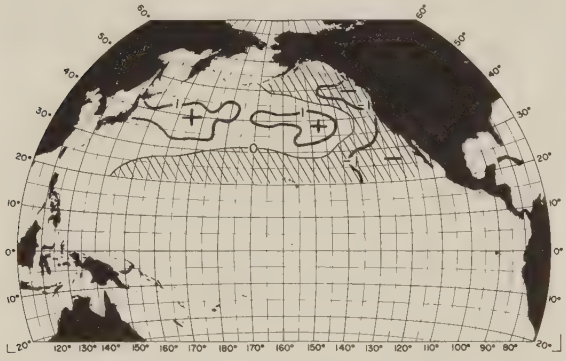

FIGURE 9.—Sea-surface temperature anomaly for November 1955. Hatched areas colder than normal. Heavy lines represent the 1° C anomaly contours which define warm (+) or cold (−) cells.

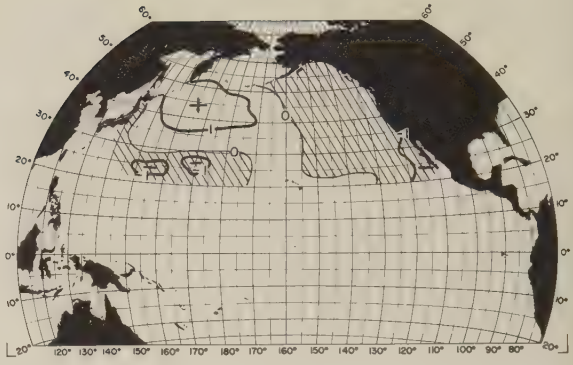

FIGURE 10.—Sea-surface temperature anomaly for February 1956. Hatched areas colder than normal. Heavy lines represent the 1° C anomaly contours which define warm (+) or cold (−) cells.

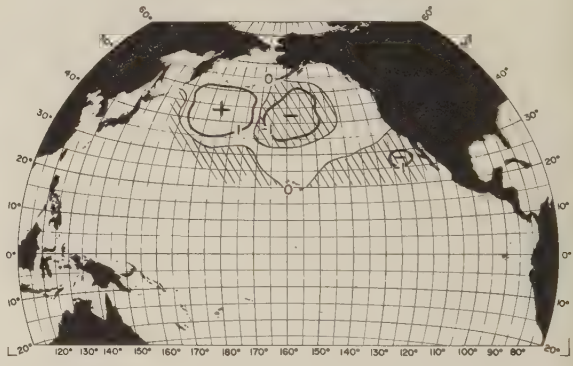

FIGURE 11.—Sea-surface temperature anomaly for August 1956. Hatched areas colder than normal. Heavy lines represent the 1° C anomaly contours which define warm (+) or cold (−) cells.

warm cell at lat 36° N to 50° N, long 175° W to 150° E was the only prominent feature in evidence and remained so until August 1956, when a large cold cell developed just east of it (Figure 11). This latter feature was short-lived, however, and during the next few months the positive area once again expanded eastward while the tendency of anomalies along the North American coast changed from variable to predominantly negative. The chart for November 1956 (Figure 12) depicts the result of this process.

Transition to a second phase in the long-term evolution of the anomaly field was foreshadowed by a small cold cell at lat 25° N to 30° N, long 160° E to 170° E (Figure 12). This cold cell grew in size but remained in about the same location through March 1957 (Figure 13) while the area of positive anomalies edged toward the North American coast. The transition was complete by June, when the cold cell shifted north and stretched eastward in a belt of negative anomalies between lat 30° N and 40° N (Figure 14). Positive values prevailed in the Gulf of Alaska and along the North American coast, effecting an almost complete reversal from the late 1955 pattern.

The negative anomalies in the west central sector, long 175° W to 160° E, contracted to form

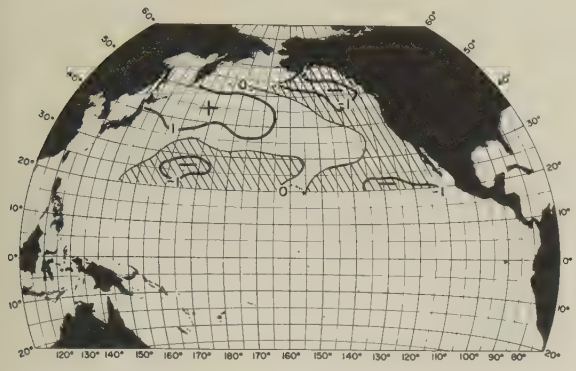

FIGURE 12.—Sea-surface temperature anomaly for November 1956. Hatched areas colder than normal. Heavy lines represent the 1° C anomaly contours which define warm (+) or cold (−) cells.

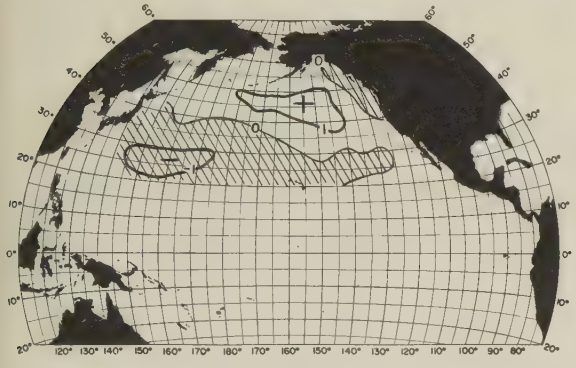

FIGURE 13.—Sea-surface temperature anomaly for March 1957. Hatched areas colder than normal. Heavy lines represent the 1° C anomaly contours which define warm (+) or cold (−) cells.

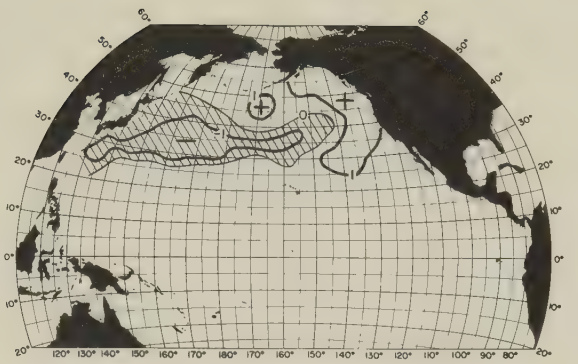

FIGURE 14.—Sea-surface temperature anomaly for June 1957. Hatched areas colder than normal. Heavy lines represent the 1° C anomaly contours which define warm (+) or cold (−) cells.

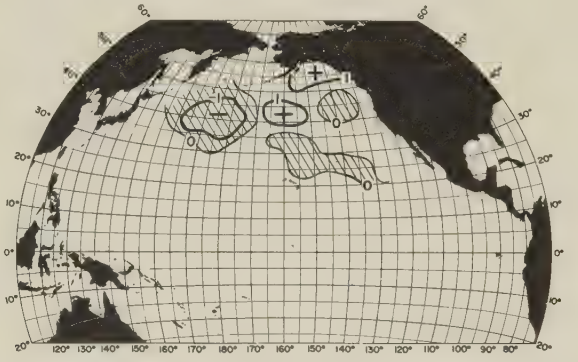

FIGURE 15.—Sea-surface temperature anomaly for August 1957. Hatched areas colder than normal. Heavy lines represent the 1° C anomaly contours which define warm (+) or cold (−) cells.

a single prominent cell which, in August 1957, was centered at about lat 40° N, long 175° E (Figure 15). This feature remained fairly steady through November 1957 (Figure 16), but all remnants of the cold anomalies in the east sector vanished.

The cell structure in the negative anomaly deteriorated at the beginning of 1958, but reformed in March with the cold cell east of its earlier position, at lat 30° N to 40° N, long 145° W to 170° W (Figure 17). Further fluctuations took place in the negative region until August 1958, when a dominant cold cell appeared at lat 35° N to 50° N, long 150° W to 175° W (Figure 18). The zone of positive anomalies along the North American coast was strongly developed during most of 1958 and 1959. However, the emphasis in the distribution shifted to the south after mid-1958, and the warm tongue reaching southwestward, south of lat 30° N, was most intense in the early months of 1959 (Figures 19 and 20).

Elsewhere the pattern tended to be somewhat weak and disorganized, and remained so until November 1959, when prominent cells, in which departures from normal exceeded 2° C, were evident in the central oceanic region of negative anomalies and in the positive coastal zone

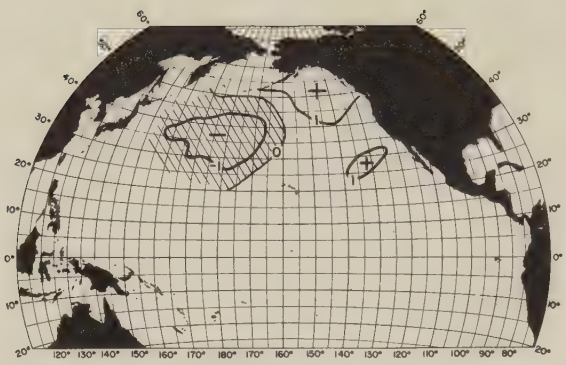

FIGURE 16.—Sea-surface temperature anomaly for November 1957. Hatched areas colder than normal. Heavy lines represent the 1° C anomaly contours which define warm (+) or cold (—) cells.

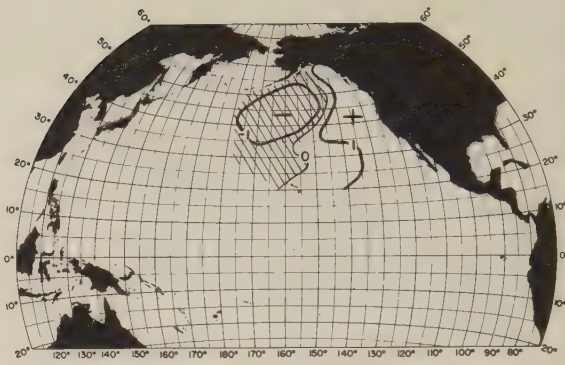

FIGURE 18.—Sea-surface temperature anomaly for August 1958. Hatched areas colder than normal. Heavy lines represent the 1° C anomaly contours which define warm (+) or (—) cells.

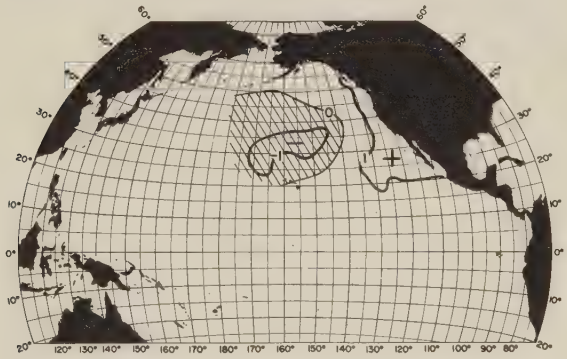

FIGURE 17.—Sea-surface temperature anomaly for March 1958. Hatched areas colder than normal. Heavy lines represent the 1° C anomaly contours which define warm (+) or cold (—) cells.

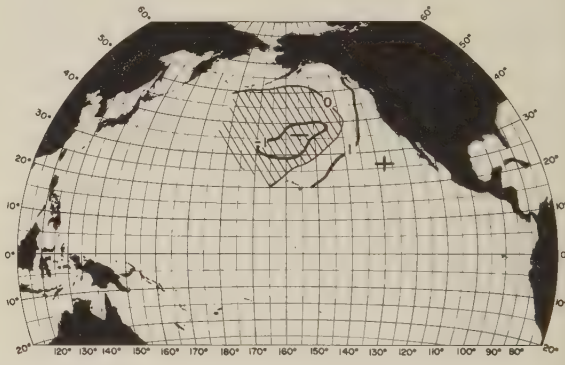

FIGURE 19.—Sea-surface temperature anomaly for January 1959. Hatched areas colder than normal. Heavy lines represent the 1° C anomaly contours which define warm (+) or cold (—) cells.

(Figure 21). The intensity and size of the warm cells diminished by February 1960 (Figure 22) and anomalies along the North American coast were generally weak, although mostly positive, through mid-1961.

The cold cell present in the west central Pacific in November 1959 had, by February 1960, pushed eastward to a new location, at lat 32° N to 48° N, long 155° E to 155° W (Figure 22). Thereafter it remained essentially within this region until the spring of 1961. However, the size and shape of the cold cell fluctuated considerably during this extended interval, as indicated in the charts for June 1960, November 1960, and February 1961 (Figures 23-25). This cold cell began to shift eastward in May 1961 and, by June, had reached a position at lat 32° N to 45° N, long 137° W to 160° W (Figure 26) where it remained through August. A new warm cell had appeared in the west sector at lat 30° N to 40° N, long 170° W to 165° E in June, but cannot easily be related to an impending transition in the anomaly field. It appeared to shift westward in July and August then vanished in September 1961, when the overall pattern collapsed.

The third and final phase in the progression of events began rather abruptly in October 1961

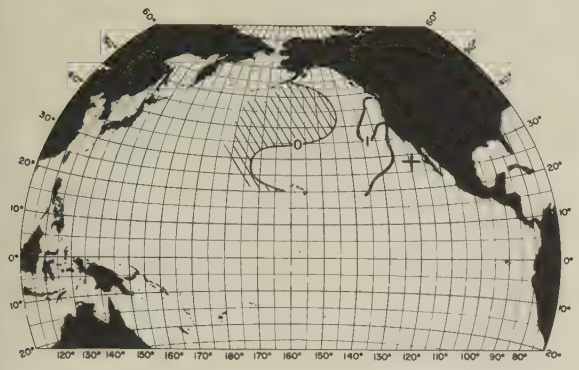

FIGURE 20.—Sea-surface temperature anomaly for March 1959. Hatched areas colder than normal. Heavy lines represent the 1° C anomaly contours which define warm (+) or cold (−) cells.

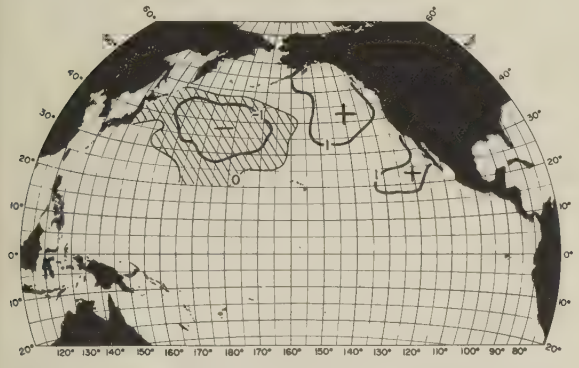

FIGURE 21.—Sea-surface temperature anomaly for November 1959. Hatched areas colder than normal. Heavy lines represent the 1° C anomaly contours which define warm (+) or cold (−) cells.

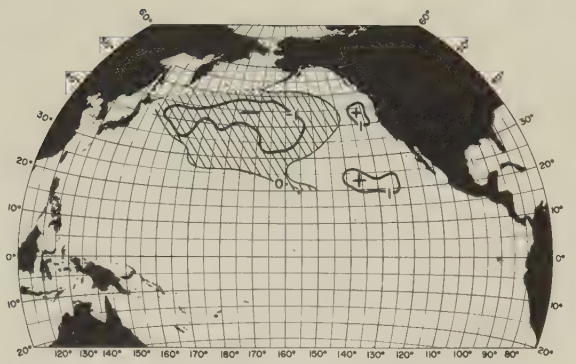

FIGURE 22.—Sea-surface temperature anomaly for February 1960. Hatched areas colder than normal. Heavy lines represent the 1° C anomaly contours which define warm (+) or cold (−) cells.

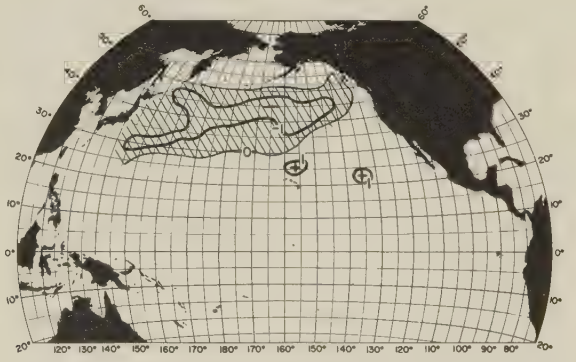

FIGURE 23.—Sea-surface temperature anomaly for June 1960. Hatched areas colder than normal. Heavy lines represent the 1° C anomaly contours which define warm (+) or cold (−) cells.

with a second reversal of the anomaly field in the central and eastern North Pacific Ocean (Figure 27). The principal feature of the new pattern was a warm cell which developed at lat 35° N to 45° N, long 145° W to 175° W. Departures from normal temperatures along the coastal zone and in the region to the southeast of the warm cell were weak but predominantly negative. In a broad sense, this distribution remained essentially undisturbed for nearly a year. The warm cell shifted eastward in January 1962 (Figure 28), diminished in size and intensity in March and April, but revived in June 1962 (Figure 29). A second warm cell was present at that time, but did not retain a separate identity for long. The original warm cell began a westward movement which, by September (Figure 30), returned it to the same location it occupied 11 months earlier in October 1961 (Figure 27).

MAINTENANCE OF PERSISTENT ANOMALIES

The examples described in the preceding section depict a recurring pattern in the distribution of sea-surface temperature anomalies. Schematically, the principal features consist of

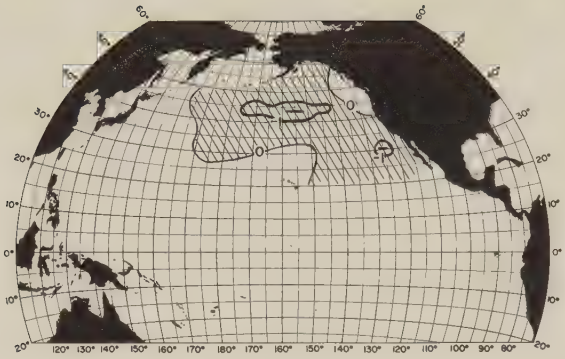

FIGURE 24.—Sea-surface temperature anomaly for November 1960. Hatched areas colder than normal. Heavy lines represent the 1° C anomaly contours which define warm (+) or cold (−) cells.

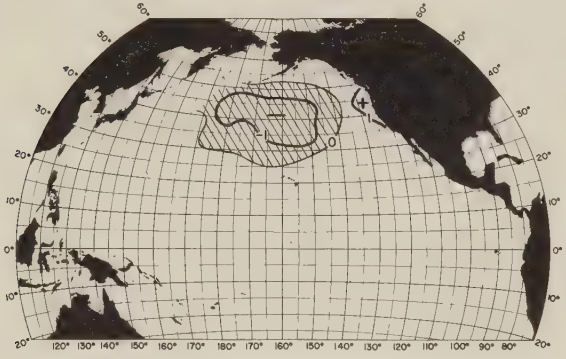

FIGURE 25.—Sea-surface temperature anomaly for February 1961. Hatched areas colder than normal. Heavy lines represent the 1° C anomaly contours which define warm (+) or cold (−) cells.

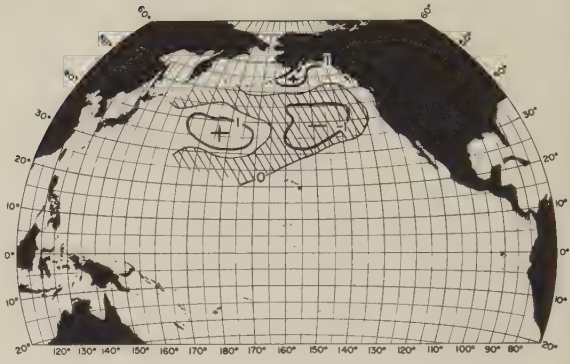

FIGURE 26.—Sea-surface temperature anomaly for June 1961. Hatched areas colder than normal. Heavy lines represent the 1° C anomaly contours which define warm (+) or cold (−) cells.

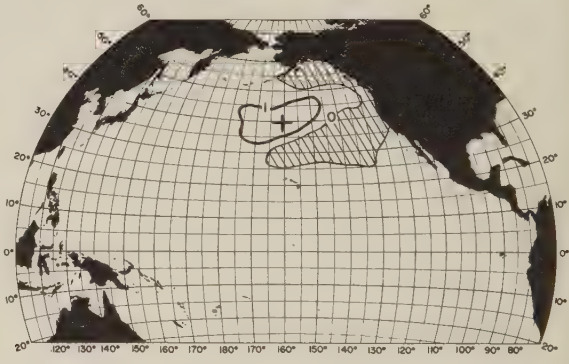

FIGURE 27.—Sea-surface temperature anomaly for October 1961. Hatched areas colder than normal. Heavy lines represent the 1° C anomaly contours which define warm (+) or cold (−) cells.

a dominant, quasi-stationary cell in a latitudinal belt of either positive or negative anomalies, located approximately between lat 30° N and 50° N, partially encircled by anomalies of opposite sign to the north, east, and south, in a zone contiguous with the North American coast. The dominant cell in this model is embedded in the North Pacific West Wind Drift Current. It reflects a wavelike displacement of the isotherms, which normally are very nearly zonal from about long 150° E to 140° W in the vicinity of lat 40° N.

The fact that this cell, which defines an area of maximum departure from normal in the temperature field, is not propagated eastward with the ocean current suggests the existence of a standing wave, or perturbation, in the current structure. Assuming this to be so, water entering the wave would turn north (or south) of its normal course, cutting across the normal isotherms and thereby causing anomalous local advection of high (or low) temperature. Downstream from the point of maximum excursion the water cuts back toward its original course and temperature conditions revert toward normal. Because the temperature gradient across the West Wind Drift Current is moderately strong, a small displacement of the isotherms

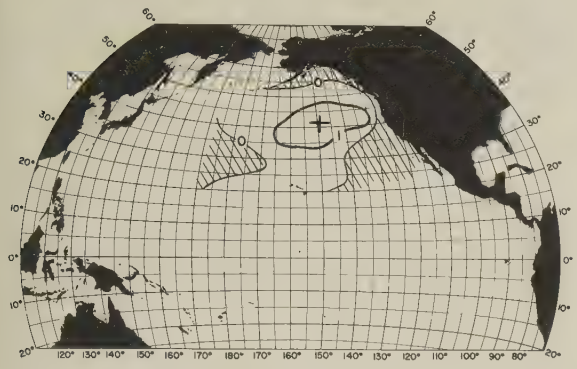

FIGURE 28.—Sea-surface temperature anomaly for January 1962. Hatched areas colder than normal. Heavy lines represent the 1° C anomaly contours which define warm (+) or cold (—) cells.

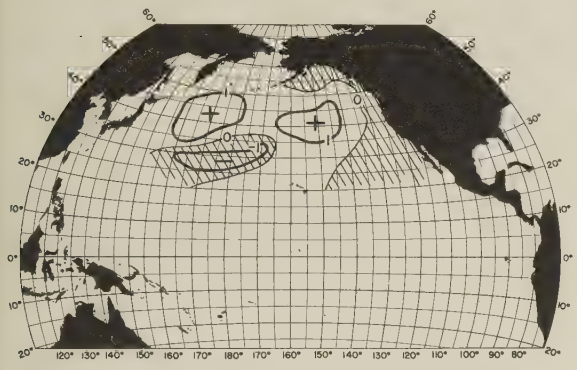

FIGURE 29.—Sea-surface temperature anomaly for June 1962. Hatched areas colder than normal. Heavy lines represent the 1° C anomaly contours which define warm (+) or cold (—) cells.

relative to the width of the current can create an anomaly of 1° C to 2° C. The amplitude of the perturbation in the current must, of course, be greater than that in the temperature field, since the water must adjust toward new equilibrium temperature appropriate to the local heat exchange processes as it changes latitude. Thus, a parcel of water following a northward deflection of the streamlines would arrive at the northern bend, or crest, of a standing wave with a temperature lower than it would have had if no deviation from zonal flow had occurred. Owing to the advective effect, it would nonetheless be warmer than an equivalent parcel of water

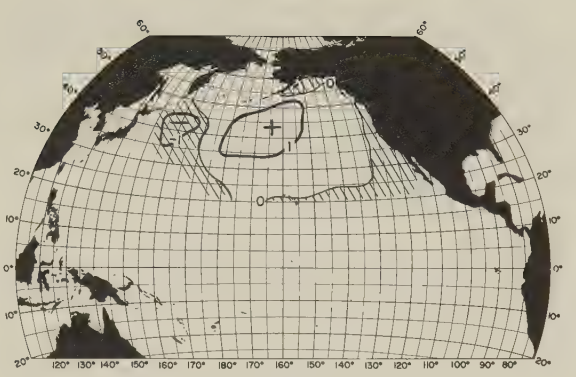

FIGURE 30.—Sea-surface temperature anomaly for September 1962. Hatched areas colder than normal. Heavy lines represent the 1° C anomaly contours which define warm (+) or cold (—) cells.

reaching the same location by a normal, zonal trajectory.

The existence of a perturbation upstream from the location where the West Wind Drift splits may have a significant effect on the proportional flow into the Alaska Gyre and the California Current. For example, in a wave formed by a northward deflection of the zonal current, water moving downstream from the crest would have a southward component which might favor more transport into the California Current. A strengthening of this current would cause the isotherms to adjust to the effects of increased cold advection and local heat exchange processes southward of their normal positions, creating a negative anomaly. Correspondingly, the reduced northward transport would decrease warm advection into the Gulf of Alaska and the isotherms would adjust to equilibrium positions farther south than normal, also creating a cold anomaly.

If the northward deflection in the foregoing example is replaced by a southward deflection, then, after passing the southern bend or trough of the wave, the water would approach the coast with a northward component, favoring more transport into the Gulf of Alaska. The balance between advection and local heat exchange would be established with the isotherms north of their normal positions, creating a warm anomaly.

In a comprehensive treatment of oceanographic survey data taken in 1955-58, Tully, Dodimead, and Tabata (1960) found the warm coastal anomaly of 1957-58 to be associated with increasing transport into the Alaska Gyre. They describe this condition in terms of a southward shift of the point of separation of flow from the West Wind Drift. From their study of the dynamic topography, they inferred that prior to the shift, in 1955 and 1956, most of the water approaching the coast south of lat 45° N entered the California Current. The fact that negative anomalies prevailed along the coastal zone in the latter half of 1955 and, to a lesser extent, in 1956 suggests that the point of separation in this period was farther north than usual.

Whether the partition of the West Wind Drift is influenced by upstream perturbations in the zonal flow structure cannot be established with certainty from the available survey data. The geopotential topography as presented by Dodimead, Favorite, and Hirano (1963) for the years 1955-59 does not reveal conclusive evidence of upstream wave structure. The physical evidence is limited to the anomalous characteristics of the surface temperature field which have already been discussed.

Some added perspective might be gained by looking briefly at the dynamic constraints applicable to a standing wave in the West Wind Drift. The average eastward current speed, U, for a wave of length L and lateral (north-south) extent D is given by Panofsky (1956) as follows:

$$U - C = \frac{2\Omega\cos\phi L^2}{4\pi^2 E}\left(\frac{1}{1 + L^2/D^2}\right)$$

where C is the wave speed (zero for a standing wave)

ϕ is the mean latitude

Ω is the angular velocity of the earth (7.292×10^{-5} sec^{-1})

E is the radius of the earth (6.37×10^{6} m).

This expression reduces to the Rossby wave equation for a uniform zonal stream on a rotating planet when the value of D approaches infinity.

In order to evaluate the right side of the above equation, we will assume dimensional similarity between the inferred wave and areas enclosed by the plus or minus 1° C anomaly contours for those cases where we presume a causal relation with the current structure. Estimates of the wave length L and of the ratio L/D were determined from rough measurements of the longitudinal and lateral extent of the warm cell present from October 1961 to September 1962. Excluding two extreme cases (April and July 1962) the longitudinal dimensions of the warm cell ranged between about 800 (March 1962) and 1600 (January 1962) nautical miles. The corresponding ratios of longitudinal to lateral extent for these particular cases were 1.3 and 2.0 respectively. Substitution of these values for L and L/D in the wave equation yields 35 and 85 cm/sec (approximately) for average current speed through a stationary wave. Of course, the areas enclosed by the 1° C anomaly contours presumably define only a portion of the hypothetical wave, and to substitute the dimensions of these areas for L and D would understate the theoretical current speed.

Current speeds in the West Wind Drift, computed from dynamic height anomalies, are generally less than 10 cm/sec (Dodimead et al, 1963). Data from drift bottles (Dodimead and Hollister, 1962) indicate current speeds up to 20 cm/sec in the same region. Thus, the theoretically computed results are too high, but considering the approximations used they are not altogether unreasonable.

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INDUCED SPAWNING OF THE NORTHERN ANCHOVY, *Engraulis mordax* GIRARD

RODERICK LEONG[1]

ABSTRACT

Anchovies were induced to mature their gonads by an artificial photoperiod of 4 hr light and 20 hr darkness at 15° C. Single injections of suspensions of salmon pituitary, carp pituitary, or a solution of human chorionic gonadotropin (HCG) promoted increase in egg diameters but did not induce spawning. Two injections, a first of HCG and the second 2 days later of either salmon pituitary or carp pituitary, induced spawning. At each spawning 6,000 to 16,000 eggs were collected, and 25% to 80% of the eggs hatched. Larvae grown from these eggs were morphologically similar to those caught in the sea.

An investigation was started at the National Marine Fisheries Service Fishery-Oceanography Center, La Jolla, Calif., in 1969, to examine methods for spawning the northern anchovy, *Engraulis mordax* Girard, under controlled laboratory conditions in order to supply eggs and larvae for experimental studies. The strategy for spawning anchovies in captivity was to provide an environment in which the fish would mature their gonads and subsequently to induce spawning through hormone treatment. The role of the environment and the use of hormone injections for inducing spawning in other species of fish has been well documented by Pickford and Atz (1957). This report describes a method for bringing the anchovy to ripeness and the effectiveness of various hormone treatments in inducing spawning. As far as is known this was the first successful attempt to artificially mature and spawn this pelagic fish in the laboratory.

MATERIALS AND METHODS

Anchovies, averaging 90 mm in length, were purchased from a San Diego bait dealer in March of 1969, transported to the laboratory, and held in circular plastic lined wading pools 1.6 m in diameter with 0.9 m of water. By the beginning of the injection trials in August of 1970, the fish had grown to an average length of 125 mm, at which length half of the fish should have been mature (Clark and Phillips, 1952). The fish were subjected to a photoperiod of 4 hr light (32 ft-c at the brightest spot on the surface of the water) and 20 hr dark (1 ft-c) for 3 months prior to the trials. Observations in the preceding year revealed that anchovies tend to mature more readily under relatively prolonged dark conditions. The tanks were constantly supplied with fresh seawater and the temperature was maintained at 15° C (Lasker and Vlymen, 1969). Much of the spawning of anchovies in nature occurs at or near this temperature (Ahlstrom, 1956). The fish were fed twice a day. In the first feeding, given at the beginning of the 4-hr light period, the fish were fed 6% of their live body weight in rotating daily rations of ground squid, ground anchovies, and brine shrimp. In the second feeding, given near the end of the light period, the fish were fed 1% of their body weight in trout chow.[2] Under this light, temperature, and food regimen approximately one-fourth of the fish developed gonads which weighed more than 6% of their body weight.

Three series of injection trials were conducted. In the first series the following dosages and types of injections were tested: 2.5, 5.0, and 10.0 mg of salmon (*Oncorhynchus tshawytscha*) pituitary[3] prepared essentially by the method of

[1] National Marine Fisheries Service Fishery-Oceanography Center, La Jolla, Calif. 92037.

[2] Ralston Purina trout chow, size 2. Reference to commercial products does not imply endorsement.

[3] Obtained through the courtesy of Dr. Irwin Haydock and the California Department of Fish and Game, Nimbus Fish Hatchery, Rancho Cordova, Calif.

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Haydock (1971), 2.5, 5.0, and 10.0 mg of commercial carp pituitary,[4] 1.0, 2.5, and 5.0 mg of deoxycorticosterone acetate (DOCA),[5] 1.0 mg of luteinizing hormone (PLH),[6] and 25, 50, and 100 international units (IU) of human chorionic gonadotropin (HCG).[5] The treatments were given in single 0.1-ml injections using a carrier of Holtfreter's solution (Emmel and Cowdry, 1964) in all cases except for DOCA where sesame oil was used. The suspensions of pituitary were prepared by triturating a weighed quantity in a small tissue grinder with enough liquid to form the proper concentration. The suspension was then pipetted into a small serum bottle where it could be withdrawn with an injection syringe. The injections were administered intraperitoneally with a 24-gauge needle between the pelvic fin and vent. Prior to injection the fish were anesthetized in 50 liters of water with 7 ppm quinaldine (Vrooman and Paloma, 1966).

Under each treatment 12 to 15 fish were injected. The sex and level of ripeness of living anchovies are difficult to distinguish and a portion of the fish in these trials were immature. Therefore it was necessary to inject this relatively large number of fish to increase the probability that some would be sufficiently developed to respond to hormone injections. Injected fish were placed in small holding tanks 1.2 $\times$ 1.2 m with 0.9 m of water. These tanks had running seawater and the temperature was also maintained at 15° C. Nets with 202-μ mesh were placed at the outflows of these tanks to collect eggs in the event of spawning. If no spawning occurred after 48 hr, the fish were stripped and fertilization attempted by the dry method (Davis, 1961). In this method the eggs and sperm are expressed, mixed, and left to stand in a dry container for 5 to 10 min before being placed in water. It was noticed in all trials that at least some males produced motile sperm.

The fish were killed after stripping and ovaries were removed from the females. Each ovary was teased apart and the major diameters of the most advanced eggs measured. The maximum diameters of injected fish were compared with the diameters of more than 500 captive uninjected females sampled during the previous 16 months to determine which of the treatments were effective in producing growth.

The effect of two injections of different hormones were examined in the second series of trials. The first injection was 50 IU of HCG and the second, given 48 hr later, was one of the following: 2.5 mg of salmon pituitary, 10.0 mg of carp pituitary, 5.0 mg of DOCA, 1.0 mg of PLH and 250 IU of gonadotropin from pregnant mare serum (PMS).[6] The methods of anesthetizing, injecting, and holding of fish were the same. If spawning did not occur within 48 hr after the second injection, the fish were stripped and fertilization attempted. In this and the final series of trials the fish were not killed after stripping and measurements of egg diameters were not made. Here the only criterion for success was spawning or production of viable eggs through stripping.

The effect of three injections of the same hormone was tested in the third and final series of trials. One group of fish was given three injections of 2.5 mg of salmon pituitary and another group three injections of 50 IU of HCG. The injections were spaced a day apart and the procedures were the same as described earlier. If no spawning occurred within 24 hr after the third injection, the fish were stripped and fertilization attempted.

RESULTS

None of the fish that were given a single injection spawned or produced viable eggs through stripping. Most of the stripped eggs were clumped, opaque, and apparently had not ovulated. Some of the treatment, however, produced noticeable increases in egg diameters. Table 1 shows the number of females under the various single-injection treatments and the number with and without eggs larger than 1.0 mm. The number of females was small in some

[4] Purchased from Stoller Fisheries, Spirit Lake, Iowa.
[5] DOCA and HCG purchased from Sigma Chemical Co., St. Louis, Mo.
[6] PLH and PMS purchased from Calbiochem, Los Angeles, Calif.

TABLE 1.—Female anchovies after various hormone injections having eggs larger than 1.0 mm in diameter. The occurrence of females with eggs larger than 1.0 mm is considered to be an indication of induced egg maturation. Of more than 500 captive uninjected fish sampled during the previous 16 months none had eggs larger than 1.0 mm. The average injected fish weighed about 25.0 g and measured 125 mm in length.

Type of injection	Dose/fish	No. females injected	No. females with eggs >1.0 mm
Salmon pituitary (*Oncorhynchus tshawytscha*)	2.5 mg	8	2
	5.0 mg	7	4
	10.0 mg	8	1
Commercial carp pituitary	2.5 mg	5	0
	5.0 mg	3	0
	10.0 mg	4	1
Human chorionic gonadotropin (HCG)	25 IU	4	1
	50 IU	8	1
	100 IU	6	0
Deoxycorticosterone acetate (DOCA)	1.0 mg	3	0
	2.5 mg	6	0
	5.0 mg	8	0
Luteinizing hormone (PLH)	1.0 mg	4	0

cases because most of the injected fish were males or had died through handling. Of the more than 500 females examined during the previous 16 months, none had eggs larger than 1.0 mm. The occurrence of females, Table 1, with eggs larger than 1.0 mm indicates that salmon pituitary, carp pituitary, and HCG are capable of promoting overnight growth of eggs. Salmon pituitary appeared to be the most potent for producing growth. The largest eggs observed were over 1.3 mm in diameter and within the size range, 1.23 to 1.5 mm, of naturally spawned eggs (Bolin, 1936). DOCA and PLH at the dosages tested were not effective in stimulating egg growth. The preponderance of fish with smaller eggs may be due to a low state of ovarian development at the time of injection.

In the second series of trials, HCG followed by DOCA, PLH, or PMS did not induce spawning and subsequent stripping produced only unovulated eggs which were not successfully fertilized. The combinations of HCG followed by salmon pituitary and HCG followed by carp pituitary induced spawning within 18 hr after the second injection. The fish spawned and fertilized the eggs themselves and large numbers of eggs were caught in the nets at the outflows of the tanks. Spawning was repeated several times with each of these two combinations of injections. The spawnings produced from 6,000 to 16,000 eggs with the percentage hatching varying from 25 to 80%. The larvae from these induced spawnings appeared morphologically normal and many were reared past 25 days by the methods of Lasker et al. (1970). The differences in the hatching percentage may be attributed to variation in the state of gonad development of parent fish at the time of injections.

The number of eggs collected suggests that only one or two females from any of the spawning groups contributed eggs. According to estimates by MacGregor (1968) female anchovies spawn almost 600 eggs per gram of fish. The average female in these trials weighed approximately 25 g and should have produced nearly 15,000 eggs. Only one or two females from any of the twice-injected groups extruded ovulated eggs upon stripping. Although the eggs were translucent and measured about 1.5 mm in diameter less than 10% hatched after being mixed with motile sperm.

In the final series of trials, three injections of salmon pituitary or three injections of HCG over a 3-day period failed to induce spawning. The stripped eggs were opaque and fertilization was not successful. These limited results suggest that the combination of HCG followed by salmon pituitary is more effective for induction of spawning than when these hormones are administered alone.

The results of these injection trials demonstrate that the northern anchovy can be induced to spawn in captivity and two effective treatments are HCG followed by salmon pituitary or HCG followed by carp pituitary after gonads are matured by a specific light-dark treatment of the fish. The induction of spawning of anchovies in the laboratory provides a practical way for supplying viable eggs for studies on larvae. In this study the time of spawning was controlled and eggs can probably be produced the year around if a large stock of fish is maintained. This was emphasized by the fact that the fish in this study were induced to

spawn during the late summer and fall months when anchovy eggs are virtually absent from the sea off San Diego. As far as is known, these are the first reported spawnings of *Engraulis mordax* in captivity and the first hormone-induced spawnings of engraulids.

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GILL RAKER APPARATUS AND FOOD SELECTIVITY AMONG MACKERELS, TUNAS, AND DOLPHINS

JOHN J. MAGNUSON AND JEAN G. HEITZ[1]

ABSTRACT

Gill raker morphology and fork length were measured from 411 fish, representing eight species of scombrids and two species of coryphaenids (dolphin). For each species linear regressions passing through the origin were determined relating mean gill raker gap in millimeters (first gill arch) with fork length in centimeters (l), and log filtering area (first gill arch) with log fork length. Mean gill raker gaps equaled: *Auxis rochei*—0.0144l, *Katsuwonus pelamis*—0.0211l, *Auxis thazard*—0.0213l, *Thunnus albacares*—0.0344l, *Thunnus alalunga*—0.0365l, *Euthynnus affinis*—0.0386l, *Thunnus obesus*—0.0391l, *Sarda chiliensis*—0.0509l, *Coryphaena hippurus*—0.0650l, *Coryphaena equisetis*—0.0655l, and *Acanthocybium solanderi*—no gill rakers. Among the species, gill raker gap was directly proportional to the number of gill rakers, but no relation occurred between mean gap and filtering areas. Gill raker gap differed markedly among species and lengths of fish. A 50-cm *K. pelamis*, a 30-cm *T. albacares*, and a 10-cm *Sarda orientalis* all had an estimated mean gap of 1 mm. Conversely the gaps of a 50-cm fish of each species were estimated to be ca. 1.0, 1.7, and 4.5 mm respectively.

Mean gill raker gaps from this study were compared with the percentage of crustaceans in stomachs of Central Pacific fishes based on literature records. Body sizes of fishes and squids in the stomachs were larger than crustaceans. Percent volumes that crustaceans contributed to the stomach content were inversely related to mean gaps (Kendall rank correlation coefficient, $\tau = -0.59$, $n = 16$, $P < 0.001$). Partial correlation indicated that gap was more important than fork length in predicting the quantity of crustaceans. Thus, the gill raker gap was related functionally with the quantity of smaller organisms in the stomachs. Presence of euphausids in stomachs of *K. pelamis* and their absence in *T. albacares* from the eastern tropical Pacific may result from the small size of euphausids and the smaller gill raker gaps of *K. pelamis* relative to *T. albacares*. Gill raker gap and the maximum distensibility of the esophagus would set physical limits on the size of food eaten. The diverse fauna assemblage of crustaceans, fishes, and squids within this size range has masked to a great extent the selective feeding that does occur among scombrids and coryphaenids on the basis of food size.

Most scombrid fishes have a varied diet that includes numerous crustaceans, cephalopod molluscs, and fishes. The Indian mackerel, *Rastrelliger kanagurta* (Cuvier), even eat phytoplankton (Bhimachar and George, 1952). The high diversity of organisms in their stomach contents has generated the opinion that scombrids are nonselective feeders, preying upon anything they encounter. Coryphaenid fishes, dolphins, eat fish predominantly.

Yet selectivity does exist in food habits of scombrids. Within a species, larger fish contain relatively fewer crustaceans and more fishes. Crustaceans constituted 44% of the stomach volume of skipjack tuna, *Katsuwonus pelamis* (Linnaeus), shorter than 50-cm fork length but only 1.5% of the volume for fish longer than 60 cm (Yuen, 1959). Similarly, crustaceans constituted 35% of the stomach volume of yellowfin tuna, *Thunnus albacares* (Bonnaterre), shorter than 130 cm but only 1% for those longer than 130 cm (Reintjes and King, 1953). Reintjes and King suggested that these differences might result, as the fish grew, from a change in food preference or a change in the ability to search out and capture larger, more mobile prey (fishes). Another consideration, in our view, is that larger predators have a reduced ability to catch small prey (crustaceans).

Prevention of food loss through the opercular gap is generally recognized as the primary function of gill rakers. Species with more closely

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spaced gill rakers are more likely to feed on plankton than those with more widely spaced rakers (Suyehiro, 1942; Yasuda, 1960a; Brooks and Dodson, 1965; Kliewer, 1970).

This paper (1) quantitatively describes the gill raker apparatus of certain scombrids and coryphaenids with respect to the gap between gill rakers and the filtering area of the first gill arch, (2) compares differences in gill raker gap among species and lengths of fish, and (3) considers the proposition that observed inter- and intraspecific variations in the diet are associated functionally with the morphometrics of the gill raker apparatus.

MORPHOMETRY OF GILL RAKER APPARATUS

Gill raker morphometry and fork length were measured from 411 fish, representing eight species of scombrids and two species of coryphaenids. Albacore, *Thunnus alalunga* (Bonnaterre), were from the commercial longline fishery operated from American Samoa, the Pacific bonito, *Sarda chiliensis* (Cuvier), were from waters off Palos Verdes, Calif., and chub mackerel, *Scomber japonicus* Houttuyn, were from the Honolulu fish market. All other specimens were from Hawaiian waters and were caught with pole and line or longline by commercial fishermen or on numerous cruises of the research vessel *Charles H. Gilbert* of the Bureau of Commercial Fisheries Biological Laboratory, Honolulu (now National Marine Fisheries Service Hawaii Area Fishery Research Center).

Measurements were from the first right gill arch of fresh or thawed specimens. The arch was removed from the fish and extended by pulling the upper and lower branches apart until the rakers were stiffly erect. Gaps between adjacent rakers (Figure 1) were measured at the base of the rakers by expanding a vernier caliper until the two gill rakers began to spread apart. Arch length and gill raker length were also measured with the caliper (Figure 1). Depending on the species, six to nine gaps and six to eight gill raker lengths spaced along the arch

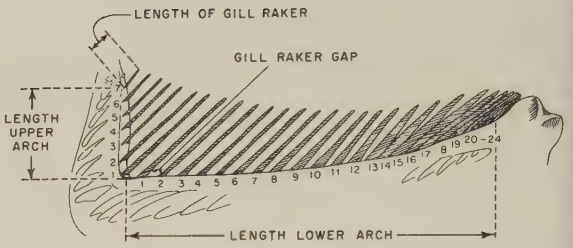

FIGURE 1.—Diagram of the first right gill arch of a scombrid as viewed from oral chamber showing the morphometric measurements. Numbers indicate particular rakers.

were obtained from scombrids and three gaps and five gill raker lengths from coryphaenids. Mean gap was the average of those measured along the arch. A gap near the middle of the lower arch was also used to represent gap width in the primary filtering area. Filtering area was calculated from average length of gill rakers and length of the arch. Lower and upper arch filtering areas were computed separately and summed.

DESCRIPTION

Gill rakers of the first arch of most scombrids were conspicuous and well developed. Inner edges of the rakers of most species were covered with numerous short, spiny protuberances. For *S. japonicus*, these spines were thin, about as long as the gill raker gap, and evenly spaced to form a finer sieve between adjacent gill rakers. The other three arches of scombrids lacked gill rakers, but smaller rakerlike processes on the inner faces of the all arches projected posteriorly to the adjacent arch forming a sieve. Inner edges of these processes had short, spiny protuberances similar to the gill rakers.

Rakers were articulated so that they became stiffly erect forming a parallel row of blade-shaped rakers when the acute angle between the upper and lower arch was expanded toward 90 degrees. In the branchial chamber the tips of the rakers extended to the inner surface of the flared gill cover.

The wahoo, *Acanthocybium solanderi* (Cuvier), has no gill rakers, but most scombrids have more than 20 elongated rakers—*K. pelamis* in

our samples had 53 to 64. Longest rakers were near the joint between the upper and lower branches of the arch. They became progressively shorter toward the ends of the arch. For example, a *K. pelamis* 50 cm long had gill rakers 21 mm long at the joint but only 2 and 8 mm at the ends of the upper and lower branches, respectively. The largest gap (1.8 mm) was near the center of the lower branch. Gaps were smaller on the upper branch than lower branch and were most narrow at the ends of the arch (0.2 mm and 0.9 mm for the upper and lower branches). Often the gap between the first raker of the upper and the first raker of lower arch was as great as the widest gap on the lower arch.

Most of the filtering area of scombrids was confined to the lower branch of the gill arch. The lower branch comprised 73 to 80% of the total. The filtering area of coryphaenids was essentially restricted to the lower arch. Dolphin, *Coryphaena hippurus* Linnaeus, had no rakers on the upper arch, pompano dolphin, *Coryphaena equisetis* Linnaeus, had only one.

Gill rakers of the two coryphaenids were shorter and more uniform in length than those of scombrids. The longest gill raker from a 55-cm *C. equisetis* was only 9 mm contrasted

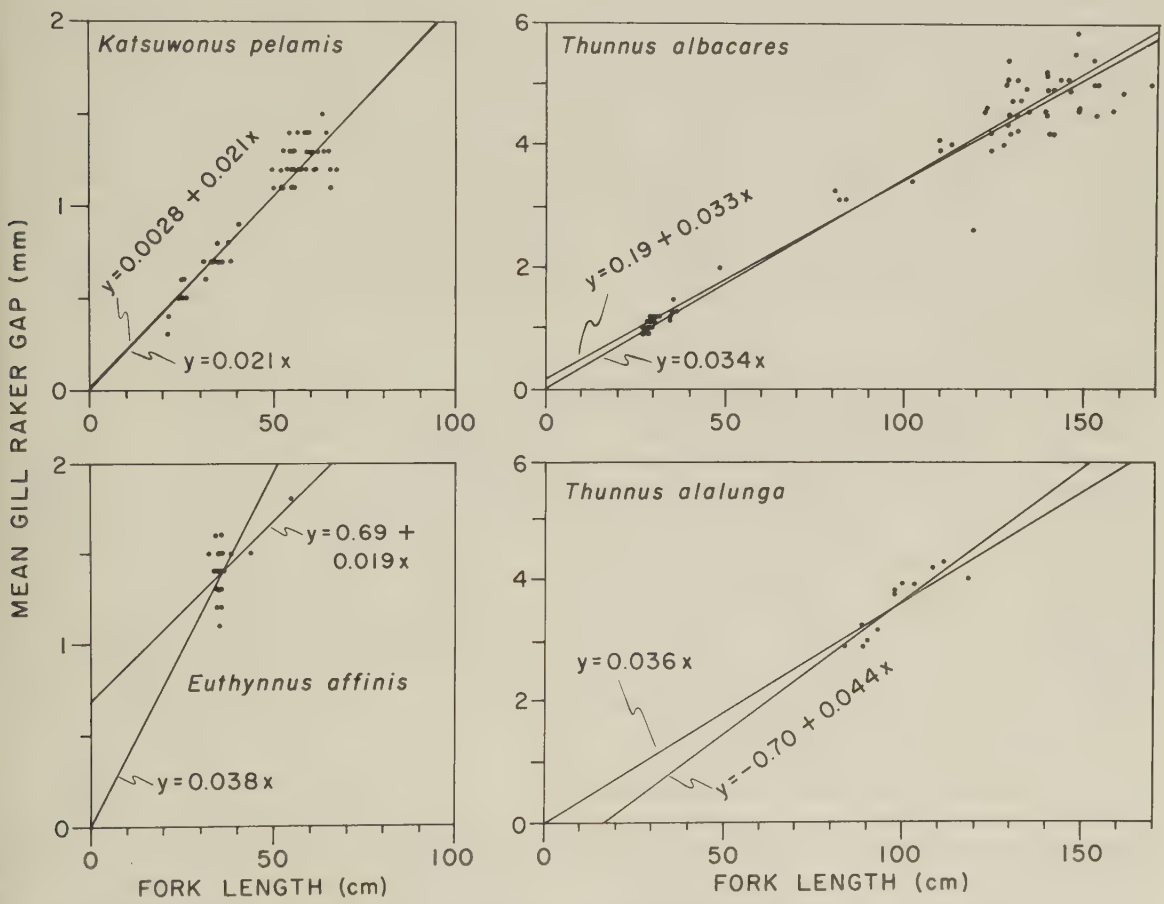

FIGURE 2.—Relation between mean gill raker gap and fork length showing the advantage of using regression through the origin for predicting mean gill raker gap especially when sample sizes are small and restricted in length range.

to 21 mm from a 50-cm *K. pelamis*. Even the longest raker of a 125-cm *C. hippurus* was only 16 mm—shorter than that of a 50-cm *K. pelamis*.

SIZE AND SPECIES COMPARISONS

METHODS

Linear regressions relating gill raker gap to fork length and log filtering area to log fork length were computed for each species. Regressions were computed once about the mean, and a second time, were forced to pass through the origin. The latter procedure was used because the ranges of fork lengths of some species were not sufficient to obtain reasonable equations (Figure 2).

Both *K. pelamis* and *T. albacares* were represented by large samples that included small and large specimens. Their regressions of gill raker gap on fork length passed close to the origin even when not forced to do so; the y-intercept was 0.00 mm for *K. pelamis* and 0.19 mm for *T. albacares* (Figure 2). In contrast, kawakawa, *Euthynnus affinis* (Cantor), and *T. alalunga* were represented by small samples that did not include small specimens. Regressions extrapolate outside the size ranges represented in our samples, the regressions forced to pass through the origin were used for all computations of gill raker gap.

The same reasoning was used for the relations between log filtering area and log fork length. In this case, the zero-zero intercept was equivalent to 1 cm fork length and 1 mm² filtering area rather than zero fork length and zero filtering area. Since most comparisons made later were for fish at least 35 cm long with filtering areas near 100 mm², errors owing to the position of the intercept were believed negligible.

SIZE AND SPECIES COMPARISONS

Linear regressions passing through the origin that relate gill raker gap to fork length and log filtering area to log fork length are presented in Table 1 along with the numbers and lengths of fishes measured.

Mean gill raker gap increased with fork length and was equal to 1.4 and 6.6% of fork length for frigate mackerel, *Auxis rochei* (Risso), and *C. hippurus*, respectively. Gill raker gap in the middle of the lower branch was usually 1.0 to 1.2 times the mean gill raker gap except for

TABLE 1.—Linear regressions passing through the origin that relate mean gill raker gap to fork length and log filtering area to log fork length and the number and length of fish measured.

Species	Number of fish measured	Regressions of gap (G) and fork length (l)	Fork length		G (mm)	Standard error of estimate for G (mm)	Regressions of log filtering area (log A) and log fork length (log l)	Mean		Standard error of estimate for Log A (mm^2)
			Mean (cm)	Range (cm)				Log l (cm)	Log A (mm^2)	
Sarda chiliensis	8	$G = 0.0509\ l$	50.13	38.7- 58.8	2.56	0.27	log A = 1.73 (log l)	1.7001	2.9246	0.0584
Auxis thazard	16	$G = 0.0213\ l$	31.40	25.1- 35.7	0.67	0.10	log A = 1.79 (log l)	1.4969	2.6730	0.0393
Auxis rochei	11	$G = 0.0144\ l$	30.24	29.2- 32.9	0.44	0.06	log A = 1.78 (log l)	1.4706	2.6291	0.0192
Euthynnus affinis	25	$G = 0.0386\ l$	36.29	33.4- 54.6	1.41	0.29	log A = 1.82 (log l)	1.5598	2.8421	0.0234
Katsuwonus pelamis	63	$G = 0.0211\ l$	47.60	21.0- 67.5	1.00	0.10	log A = 1.83 (log l)	1.6776	3.0135	0.0697
Thunnus alalunga	12	$G = 0.0365\ l$	98.56	84.0-118.8	3.59	0.25	log A = 1.81 (log l)	1.9937	3.6088	0.0343
Thunnus albacares	74	$G = 0.0344\ l$	94.86	27.1-166.9	3.31	0.36	log A = 1.78 (log l)	1.9771	3.3468	0.0405
Thunnus obesus	82	$G = 0.0391\ l$	132.96	75.2-175.3	5.26	0.60	log A = 1.85 (log l)	2.1236	3.9058	0.0449
Coryphaena equisetis	38	$G = 0.0655\ l$	94.90	30.8- 58.7	6.16	0.54	log A = 1.39 (log l)	1.9773	2.7339	0.0704
Coryphaena hippurus	68	$G = 0.0650\ l$	40.52	63.2-126.4	2.69	0.34	log A = 1.36 (log l)	1.6077	2.1716	0.0727

of gill raker gap on fork length for these two species did not closely approach the origin (Figure 2); we believe these equations would also have had y-intercepts near 0.0 mm if lengths of our specimens had been more evenly distributed. Since some comparisons were made that *A. rochei* (1.3) and *K. pelamis* (1.4). Mean gap increased in direct proportion to fish length; i.e., if length doubled, gap also doubled.

Filtering area increased as the 1.4 to 1.8 power of fork length. When these regressions were not forced to pass through the origin, the filter-

ing area increased as the 2.2 power of fork length for *K. pelamis* and the 1.9 power for *T. albacares*. Forcing the regressions to pass through the origin may have decreased the slope.

To facilitate comparison of different species, the mean gap and filtering area were computed from the regression in Table 1 for fish with a fork length of 35 cm. These are listed in Table 2 in order of decreasing number of gill rakers, increasing gap, and decreasing filtering area.

As expected, the number of rakers and gill raker gap were closely related (Table 2). Lack of complete correspondence may have resulted from differences in the thickness of gill rakers, differences in the length of the gill arch, or both.

Among scombrids no relation was evident between filtering area and number of rakers or between filtering area and mean gill raker gap (Table 2). Apparently, the length of raker was an important variable determining differences

TABLE 2.—Scombrid and coryphaenid species (35-cm fork length) listed in order of increasing numbers of gill rakers, and decreasing mean gill raker gap and filtering area.
(Data on *S. orientalis* from one fish, *S. japonicus* from two fish.)

Rank	Mean number of rakers (*n*)	Species	Mean gill raker gap (*mm*)	Species	Filtering area (mm^2)	Species
12	8	*Coryphaena hippurus*	3.3	*Sarda orientalis*	--	*Scomber japonicus*
11	10	*Coryphaena equisetis*	2.3+	*Coryphaena equisetis*	685	*Thunnus obesus*
10	11	*Sarda orientalis*	2.3—	*Coryphaena hippurus*	650	*Euthynnus affinis*
9	25	*Sarda chiliensis*	1.8	*Sarda chiliensis*	620	*Thunnus alalunga*
8	26	*Thunnus obesus*	1.4	*Thunnus obesus*	530	*Katsuwonus pelamis*
7	29	*Thunnus alalunga*	1.4	*Euthynnus affinis*	570	*Auxis thazard*
6	30	*Thunnus albacares*	--	*Scomber japonicus*	550	*Auxis rochei*
5	31	*Euthynnus affinis*	1.3	*Thunnus alalunga*	450	*Sarda chiliensis*
4	37	*Scomber japonicus*	1.8	*Thunnus albacares*	410	*Thunnus albacares*
3	40	*Auxis thazard*	0.74	*Auxis thazard*	--	*Sarda orientalis*
2	45	*Auxis rochei*	0.74	*Katsuwonus pelamis*	135	*Coryphaena hippurus*
1	58	*Katsuwonus pelamis*	0.51	*Auxis rochei*	120	*Coryphaena equisetis*

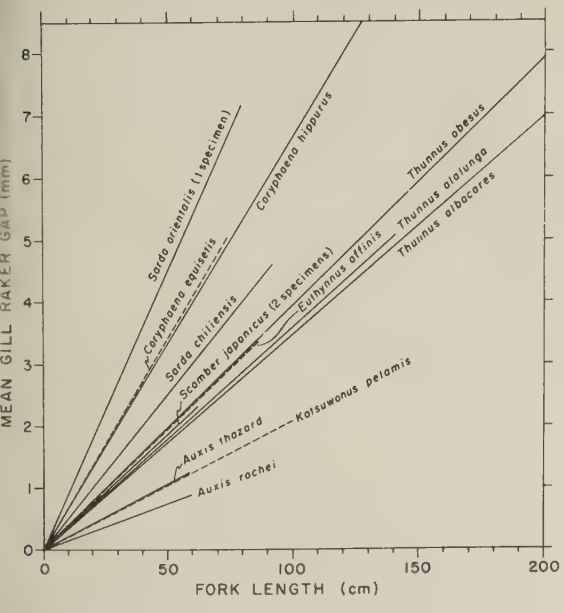

FIGURE 3.—Comparison of the mean gill raker gap and fork length relationship for various scombrid and coryphaenid fishes. Lengths shown approximate ranges known for each species.

in filtering area among species. Coryphaenids had a larger gill raker gap and smaller filtering area than any scombrid except striped bonito, *Sarda orientalis* (Temminck and Schlegel).

Among scombrids 35 cm long, *Sarda* had the largest gaps (1.8-3.3 mm) and *Auxis* and *Katsuwonus* the smallest (0.51-0.74 mm). *Thunnus*, *Euthynnus*, and *Scomber* had intermediate gap widths (1.2-1.4 mm). Among *Sarda*, *Auxis*, and *Thunnus* represented in our samples, species within genera had more similar gill raker gaps than those in different genera. On this basis alone food habits for fish of the same length would be expected to be more similar within genera than among genera.

Mean gill raker gap differed markedly with species and length of fish (Figure 3). For example, a 50-cm *K. pelamis*, a 30-cm *T. albacares*, and a 10-cm *S. orientalis* all had a mean gill raker gap of approximately 1 mm. Conversely, gill raker gaps of these three species differed markedly at the same fork length. Gaps of 50-cm *K. pelamis*, *T. albacares*, and *S. orientalis* were ca.

TABLE 3.—The average size of individual crustaceans, squids, and fishes in the stomachs of scombrids from the central Pacific.

Species	Volume of individual organisms (ml)			Volume of food analyzed (ml)	Source
	Crustaceans	Squids	Fishes		
Thunnus albacares	0.3	4.8	6.4	44,680	King & Ikehara (1956)
Thunnus obesus	0.6	9.5	8.2	22,297	King & Ikehara (1956)
Thunnus albacares	0.2	3.8	4.6	52,336	Reintjes & King (1953)
Katsuwonus pelamis	0.2	4.3	3.7	13,974	Waldron & King (1963)
Mean (unweighted)	0.3	5.6	5.7		

1.0, 1.7, and 4.5 mm, respectively. Selectivity of the gill raker apparatus would vary with gill raker gap, a function of both species and length of the fish. Thus, a small *T. albacares* and a large *K. pelamis* should have more similar diets than a small and a large *T. albacares*. Any number of such predictions can be generated from Figure 3. A fish with smaller mean gap, regardless of its species or length, would be expected to be more planktivorous.

RELATION BETWEEN GILL RAKER GAP AND DIET

Stomach-content data from published literature from the central Pacific were compared with the mean gill raker gaps reported here to test the hypothesis that fish with a finer gill raker gap have a greater proportion of smaller organisms (crustaceans) in their diet.

Crustaceans in the diet of scombrids from the central Pacific were smaller than were the other major food organisms (squids and fishes) (Table 3). The volume of individual, partially digested crustaceans in the stomachs of five species averaged 0.3 ml whereas individual, partially digested squids and fishes averaged 5.6 and 5.7 ml, respectively. The much smaller body size of the crustaceans was not likely the result of differential digestion, especially since the exoskeleton of crustaceans, if anything, might be expected to slow, rather than accelerate, digestion (Pandian, 1967).

For comparison with gill raker data, the percent volumes of the stomach content comprised by crustaceans, squids, or fishes are presented in Table 4 for five scombrids and one coryphaenid. Only stomach data from the central Pacific were used because differences in typical body size of crustaceans in scombrid stomachs from other regions would have invalidated these

TABLE 4.—Food of scombrid and coryphaenid fishes from the central Pacific divided into the percentages of the stomach volume that were crustaceans, squids, or fishes. The median fork length of fishes in the sample is also given along with the literature source for the data.

Species	Stomach content (*percent* volume)			Fork length		Number of stomachs	Literature source
	Crustaceans	Squids	Fishes	Median (*cm*)	Range (*cm*)		
Acanthocybium solanderi	0	0	100	111	104-123	3	Tester & Nakamura (1957)
Acanthocybium solanderi	0	--	--	--	54-198	235	Iversen & Yoshida (1957)
Euthynnus affinis	8	0	92	49	31-67	32	Tester & Nakamura (1957)
Katsuwonus pelamis	44	--	40	44	39-49	>25	Yuen (1959)
Katsuwonus pelamis	4.0	23	72	47	33-60	305	Waldron & King (1963)
Katsuwonus pelamis	67	7	26	50	40-61	67	Tester & Nakamura (1957)
Katsuwonus pelamis	25	--	70	55	50-60	>25	Yuen (1959)
Katsuwonus pelamis	3.7	19	74	73	60-89	254	Waldron & King (1963)
Katsuwonus pelamis	1.5	--	91	73	62-84	>25	Yuen (1959)
Thunnus albacares	45	14	33	80	53-100	544	Reintjes & King (1953)
Thunnus albacares	39	9	49	115	100-130	205	Reintjes & King (1953)
Thunnus albacares	1.7	29	65	135	85-140	188	King & Ikehara (1956)
Thunnus albacares	3	4	93	140	130-168	26	Reintjes & King (1953)
Thunnus albacares	0.8	30	60	148	140-175	251	King & Ikehara (1956)
Thunnus obesus	2.3	26	70	128	75-140	63	King & Ikehara (1956)
Thunnus obesus	1.4	36	58	158	140-200	103	King & Ikehara (1956)
Coryphaena hippurus	1.6	2	97	81	42-121	52	Tester & Nakamura (1957)

TABLE 5.—Percent crustaceans by volume in the stomachs, median fork length, mean gill raker gap, and species of fish. (Ranks are from smallest to largest and ordered by the percentage of crustaceans in the stomachs.)

Species	Crustacea		Median fork length		Mean gill raker gap	
	%	Rank	(cm)	Rank	(mm)	Rank
Acanthocybium solanderi	0	1	111	10	0	16
Thunnus albacares	0.8	2	148	15	5.2	13
Thunnus obesus	1.4	3	158	16	6.3	15
Katsuwonus pelamis	1.5	4	73	6	1.5	5
Coryphaena hippurus	1.6	5	81	9	5.3	14
Thunnus albacares	1.7	6	135	13	4.7	10
Thunnus obesus	2.3	7	128	12	5.1	12
Thunnus albacares	3	8	140	14	4.9	11
Katsuwonus pelamis	3.7	9	73	6	1.5	5
Katsuwonus pelamis	4.0	10	47	2	0.99	2
Euthynnus affinis	8	11	49	3	1.9	7
Katsuwonus pelamis	25	12	55	5	1.2	4
Thunnus albacares	39	13	115	11	4.0	9
Thunnus albacares	44	14	80	8	2.8	8
Katsuwonus pelamis	45	15	44	1	0.92	1
Katsuwonus pelamis	67	16	50+	4	1.0	3

analyses. The galatheids and portunids dominating the crustaceans found in *T. albacares* stomachs in the eastern tropical Pacific (Alverson, 1963) are much larger (Longhurst, 1967; Jerde, 1967b) than the typical crustaceans from the stomachs of central Pacific scombrids given in Table 3. Also, data were not used if fewer than 25 stomachs had been examined. None of the 238 *A. solanderi* contained crustaceans and 0% crustaceans in the stomach was considered a reasonable estimate for any larger *A. solanderi*. The median or midrange fork length of fish was determined for each set of stomach data. Then mean gill raker gaps for fish of those species and length were estimated with the regressions from Table 1. Data on median fork length, mean gill raker gap, and percent crustaceans by volume in the stomach are presented in numerical and ranked form in Table 5.

Percent volumes that crustaceans contributed to the stomach content were inversely related to mean gill raker gap (Figure 4a) (Kendall rank correlation coefficient, $\tau = -0.59$; $n = 16$; $P < 0.001$) and to fork length (Figure 4b) (Kendall rank correlation coefficient, $\tau = -0.45$; $n = 16$; $P < 0.01$). Several notable exceptions occurred in the relation with fork length (Table 5, Figure 4b). *C. hippurus*, 81 cm long, contained 2% crustaceans while *T. albacares*, 80 cm long, contained 45% crustaceans. *T. albacares*, 135 cm long, also contained 2% crustaceans. Not unexpectedly, *C. hippurus*, 81 cm long, and *T. albacares*, 135 cm long, both had mean gill raker gaps near 5 mm whereas the 81-cm *T. albacares* had a smaller mean gill raker gap near 3 mm. The somewhat closer correspondence of percentage of crustaceans to gill raker gap than to fork length can be observed by comparing Figures 4a and 4b or by comparing the associated probabilities of no correlation ($<.01$ versus $<.001$).

Kendall partial rank correlation coefficients were computed to determine the association between percent crustaceans in the stomach and gill raker gap, with the effect of fork length held constant. The partial correlation coefficient between percent crustaceans and gap, independent of variation in fork length, was —0.43 while the partial correlation between percent crustaceans and fork length independent of variations in gap, was only —0.05. Thus, although fork length was correlated with the percent crustaceans, this correlation resulted from the association between gill raker gap and fork length. Gill raker gap was the important variable correlated to percent crustaceans in the diet.

Data on percent crustaceans in the stomach by volume were also presented for *K. pelamis* and *T. albacares* of various size by Alverson (1963) and for *K. pelamis* and blackfin tuna, *Thunnus atlanticus* (Lesson), by Suarez Caabro

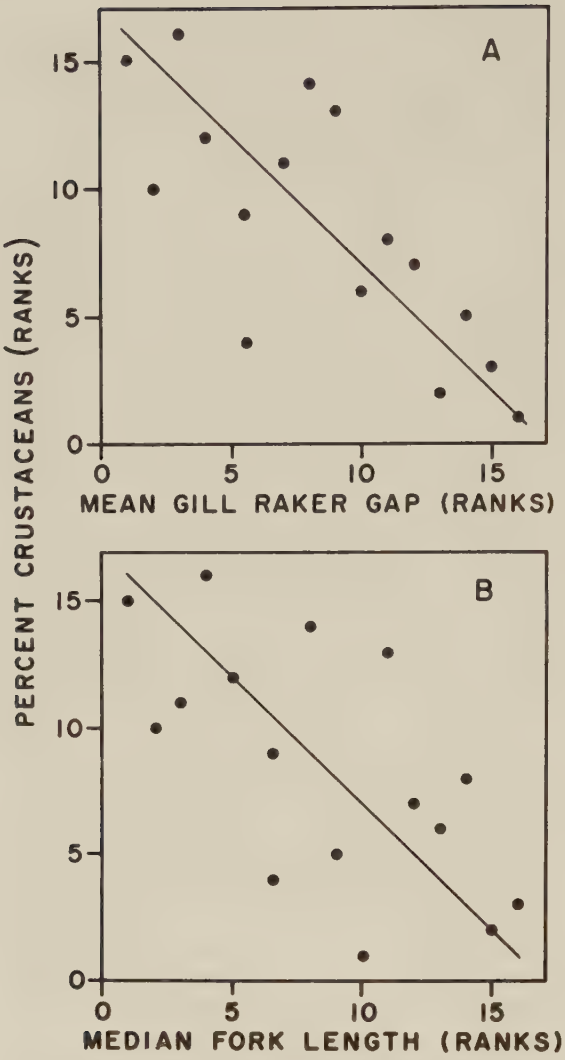

FIGURE 4.—Relation between percentage crustaceans by volume of diet (ranked) and the (a) mean gill raker gap of a fish (ranked) and (b) fork length of fish (ranked). The diagonal line depicts a perfect inverse relationship.

and Duarte Bello (1961). These were not used in the present analysis because sample sizes were fewer than 25 fish or because the size of the individual crustaceans was unavailable. Regardless, larger *K. pelamis* in both studies contained less crustaceans. However, larger *T. albacares* in Alverson's study tended to eat more crustaceans than did smaller specimens. This may have been because the crustaceans in their diet were relatively large.

Alverson's paper also presents a good example of selectivity among crustaceans that may be based on size of gill raker gaps. *K. pelamis* and *T. albacares* from the same areas had markedly different diets. Crustaceans contributing the greatest volume to the stomachs were galatheids and portunids for *T. albacares* but euphausids for *K. pelamis*. Euphausids were rare in stomachs of *T. albacares* even when common in the micronekton (Blackburn, 1968). Galatheids and portunids (Longhurst, 1967; Jerde, 1967b) are typically larger in size than euphausids (Jerde, 1967a). The small euphausids were not important in stomachs of *T. albacares* (i.e., 1% of the volume) in any of the areas of the eastern tropical Pacific studied by Alverson (1963), but the larger galatheids and portunids were important in the stomachs of *K. pelamis* from certain areas. The above observations would be the predictions from gill raker gaps—*T. albacares* have broader gaps than *K. pelamis* and would not be expected to capture the smaller crustaceans.

The major hypothesis under investigation in the present study was that the quantity of smaller organisms (crustaceans) eaten should be related to the selectivity of the gill raker apparatus. The above correlations on central Pacific data, although only crude in nature, lend support to this idea. A more definitive test would require extensive data on the size of food organisms and the diet of scombrids over more narrow length ranges than are available from the published literature.

Even though the structure of the gill raker apparatus ultimately determines the smallest size of prey, it is possible that actual selection of fishes is made prior to ingestion (Ivlev, 1961; Galbraith, 1967). Galbraith believed that the gill rakers of yellow perch, *Perca flavescens* (Mitchill), and rainbow trout, *Salmo gairdneri* Richardson, could have retained smaller zooplankton than were typically found in their stomachs. These species ate only larger *Daphnia* even though numerous smaller ones were in the zooplankton.

Several authors have pointed out that fish tend to select the largest food organisms available to them (Hayashi, 1956, as cited in Yasuda, 1960b; Ivlev, 1961; Brooks, 1968). The large mouth of larval scombrids facilitates capture of large copepods at first feeding and contributes to their rapid early growth rates (Shirota, 1970). The responsiveness of at least one scombrid to food is influenced by the size of the food organism—*K. pelamis* ate whole shrimp and squid at the beginning of a feeding, but as they became sated, they would only eat cut-up pieces of smaller size (Nakamura, 1962). Feeding behavior of Atlantic mackerel, *Scomber scombrus* Linnaeus, (Sette, 1950) and northern anchovy, *Engraulis mordax* Girard, (Leong and O'Connell, 1969) changes with the size of food. When small food is present, they open the mouth wide and flare the opercles in a filter feeding mode, but with larger food they make individual biting attacks. *S. japonicus* eats food smaller than would be predicted by gill raker gap (Hiyama and Yasuda, 1957). The spiny process we observed on the rakers of *S. japonicus* probably form an even finer sieve than is formed by the rakers themselves. Regardless of the mode of selection (anatomical, behavioral, or perceptual), the selective capabilities of scombrids and coryphaenids would appear to be correlated with the anatomy of the gill raker apparatus.

An individual scombrid is able to prey on organisms differing greatly in size. It is capable of engulfing and retaining crustaceans, small fishes, and squid by means of a well-developed gill raker apparatus. It is also capable of pursuing, capturing, and ingesting fast-moving fishes and squids, provided they are not too large to be swallowed whole. The gill raker gap and maximum distensibility of the mouth and esophagus then would be expected to set limits on the range of food sizes eaten by scombrids. Within this size range a diverse faunal assemblage exists in the sea that includes numerous species of crustaceans, fishes, and molluscs. The diversity of species in the size range consumed by an individual scombrid has, to a great extent, masked the selectivity that does occur. The present paper provides some evidence for selection of organisms above a minimum size determined by the magnitude of gill raker gaps.

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NATURE OF FREE RADICALS IN FREEZE-DRIED FISHERY PRODUCTS AND OTHER LIPID-PROTEIN SYSTEMS

WILLIAM T. ROUBAL[1]

ABSTRACT

The electron paramagnetic resonance spectrometer makes it possible to detect and study radicals which are produced during lipid peroxidation in freeze-dried lipid-protein systems. In such systems, two general types of resonances are observed. By studying effects of added antioxidants, added autoxidizable lipid, and type of substrate, it is possible to differentiate and characterize the two signals which are observed. It is postulated that the immobilized radicals observed in dry systems are the same as those that are responsible for damage at the molecular level in oxygenated lipid-protein-water emulsions, stored meats of normal moisture content, and in antioxidant deficiency states in man and animals.

Fish from the sea are rich in polyunsaturated fatty acids. Furthermore, when fish are processed as a foodstuff, the unsaturated fatty acids will, if unprotected, readily undergo lipid peroxidation.

In the presence of proteins, enzymes, nucleotides and other classes of biological materials, lipid peroxidation is radiomimetic, that is, it produces similar if not the same effects as ionizing radiation—a damage mechanism which is known to be mostly free radical in nature. Accordingly, such materials as freeze-dried fish tissue, dried fish meals, and protein concentrates incompletely freed of residual unsaturation, are all prone to undergo various deteriorative changes as a consequence of lipid peroxidation.

Although much emphasis has been placed in the past, and continues to be at present, on the participation of radicals in events leading to damage, radicals of the oxy or peroxy type have not been directly characterized by electron paramagnetic resonance (EPR) spectroscopy in living systems or in emulsions for in these the steady state concentration of radicals is universally low. Nevertheless, the EPR method remains as the one method best suited for characterizing radicals.

This paper discusses recent research employing systems which, for the first time, are favorable for the detection and study of EPR signals which arise with the onset of lipid oxidation. Mechanisms for the formation of radicals as well as reactions of radicals themselves are discussed.

MATERIALS AND METHODS

Freeze-dried and isopropyl-extracted rockfish myofibrillar protein, and freeze-dried rockfish sacroplasmic protein were provided by the NMFS Technological Laboratory in Seattle. In addition, a polyunsaturated fatty acid (PUFA) concentrate (75% C22:6 + 25% C22:5) was also provided by this laboratory. Freeze-dried human serum albumin (Grade III) and bovine serum albumin (BSA; crude powder) were obtained from Sigma. Freeze-dried silver salmon light flesh was prepared from a slurry of fresh fillet. All freeze-dried materials were stored at —60° C in the dark under nitrogen prior to exposure to air. Lipid-protein mixtures were prepared merely by thoroughly mixing the C22:6 concentrate with protein, usually in a ratio of 2:1 (protein to lipid) by weight. All oxidations were conducted in air at room temperature. All EPR studies were conducted at room temperature according to the procedures of Roubal (1970).

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RESULTS AND DISCUSSION

ELECTRON MIGRATION IN PROTEINS

As a base for comparing EPR signals in processed food materials, it will be instructive at this point to consider briefly the mechanism(s) which may be operative (disregarding for the moment, lipid oxidation) in the production of the observed signals in *carefully* freeze-dried tissue. In recent years, considerable attention has been given to the idea of the migration of energy over comparatively long distances in cells; such transfer of electrons is implicated in the process of mutation by ionizing radiation, in the process of nerve conduction, and in certain biochemical photo dissociations. There are problems of a theoretical nature associated with such hypotheses, and these are reviewed in the discussions of Blyumenfel'd (1957).

Terenin (1947) and Terenin and Krasnovskii (1949) have criticized Szent-Györgyi's hypothesis that electrons move along the protein polypeptide backbone in conductivity bands analoguous to the movement of electrons in conductivity bands of semiconducting metals. Although polypeptide chains exhibit properties reminiscent of conjugated unsaturation, Terenin poses the interesting observation that some 70 kcal/mole, a large amount of energy, would be required to mobilize electrons; evidently at usual temperatures, conductivity bands are empty.

Notwithstanding the fundamental objections to energy migration in native proteins, Blyumenfel'd (1957) considered the participation of triplet states in energy transfer, a possibility that is partially confirmed by the fact that the phosphorescence spectrum of proteins lies in the region of 4000 Å and corresponds closely to the calculated excitation energy which would be sufficient for energy transfer across molecular orbitals originating within the framework of hydrogen bonds. However, in the absence of prosthetic groups, it seems reasonable that few, if any, electrons would be free for mobilization. When prosthetic groups are present, however, and when they lie close to those of the protein (energetically speaking), electrons could be transferred into low lying orbitals of protein. Once in these molecular orbitals, the forbidden triplet transition to the singlet ground state would tend to maintain electrons in the triplet state energy levels. Under these circumstances, the electron would move along a chain of peptide hydrogen bonds until disposed of into some other favorably bound group.

RADICAL CONTENT IN PROCESSED FISHERY MATERIALS

For conventionally processed foodstuffs, tissues, and meals (that is, solvent-extracted proteins or proteins processed at room temperature), the situation is quite different. No longer do we have a protein substance identical in character to the native material; bonds have been broken, and the original geometrical arrangement of the protein chains has been completely disrupted. In many instances, certain compounds or classes of compounds have been selectively removed while at other times other substances are purposely added back to the protein.

Interestingly, the present study has shown that such materials as protein concentrates containing some residual lipid, fish meal, and freeze-dried tissue samples not processed with utmost caution in the freezing and freeze-drying steps, all exhibit characteristic EPR signals when exposed to air. Indeed, it is generally true that the radical content of haphazardly handled materials is usually higher than for an equal weight of similar material which has been processed by careful handling, freezing in liquid nitrogen, and careful removal of water. Unable to migrate along conductive pathways, effective charge transfer to radicals from donor molecules or reactants is apparently reduced (thus the radicals act as though caged or matrixed) and no longer do radicals interact freely with one another nor do they react at once with other cellular constituents. However, the efficiency in the reduction of charge transfer undoubtedly depends on the nature of the sample and its treatment. Just how immobilized such radicals are is open to conjecture. They are im-

mobilized sufficiently, however, so as to be detectable for fairly long periods of time.

CHARACTERISTIC FORM AND APPEARANCE OF EPR SIGNALS OBSERVED IN DRIED PRODUCTS

Although a high resolution EPR analysis can usually be performed with dilute solutions of soluble, low molecular weight organic radicals, the same is seldom true for powdered samples, and especially for powdered samples of complex molecules. The requirements for resolution are: magnetically dilute systems (in order to prevent spin-spin interaction), long relaxation times, and a low rf field. In the solid state, relaxation times are shortened because of the more effective coupling between spin states and the surrounding lattice—cooling the sample (perhaps to the temperature of liquid nitrogen or below) will often increase the relaxation time to acceptable values. Related to relaxation is the line broadening arising with molecular dipole interactions. The notable example is the so-called "oxygen effect"—some radicals will be far removed from the magnetic influences of the molecular oxygen di-radical while other free radicals in the sample will be near oxygen molecules. Consequently, free radicals of the sample will experience a variety of magnetic fields, producing a collective band of resonances resulting from the distribution of collective magnetic fields superimposed on the external instrument magnetic field. Therefore, in solid state studies, radicals, because of their random alignment, exhibit anisotropic coupling which broadens the lines and makes interpretation difficult. Spectral features which can be used to characterize the radicals are the measurement of the g-value, line shape, and changes in these parameters upon chemical or physical treatment of samples.

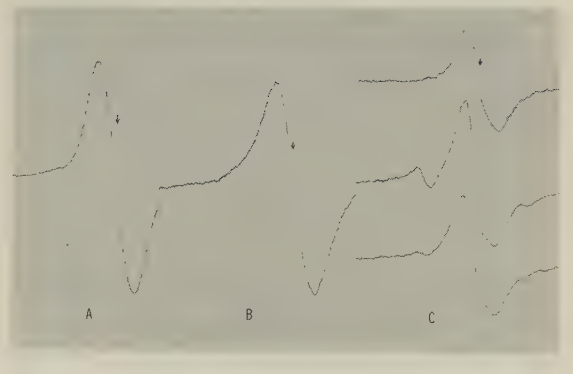


FIGURE 1.—EPR spectra for protein essentially free of lipid and for a lipid-treated protein, all exposed to air. A. Freeze-dried and solvent extracted rockfish myofibrillar protein. B. Freeze-dried human serum albumin. C. Crude bovine serum albumin (BSA) (upper trace). Crude BSA + C22:6 fatty acid (2:1 by wt) oxidized in air at room temperature for 2 hr (middle trace). Same material in air at room temperature at the end of 4 hr (lower trace). The arrows denote the $g = 2$ or free-spin value.

FIGURE 2.—EPR lipid signals in marine protein concentrates exposed to air. A. Freeze-dried rockfish flesh exposed to air for 20 hr at room temperature. B. Commercially available FPC, now 2 years old, low lipid initially, which still exhibits a weak lipid signal. C. Freeze-dried silver salmon light flesh exposed to air for 10 hr at room temperature. Arrows denote $g = 2$.

EPR SPECTRA OF FISHERY PRODUCTS

Shown in Figures 1, 2, and 3 are EPR signals which are observed in lipid-protein models and in dry ice-frozen, freeze-dried fish tissue, all of which are under investigation in this laboratory. As with carefully freeze-dried samples of the type discussed above (liquid nitrogen frozen and freeze-dried), the resonances are devoid of hyperfine structure of the type normally

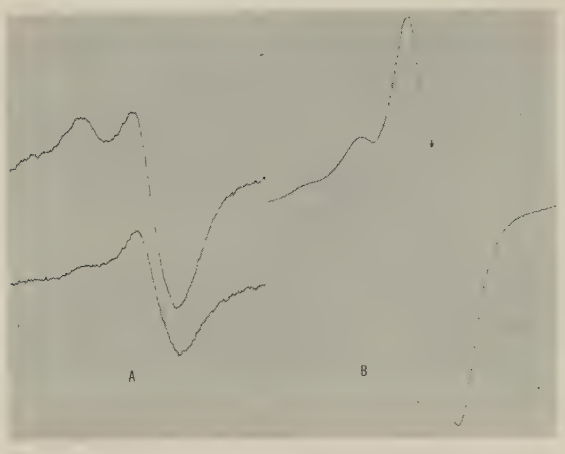


FIGURE 3.—EPR lipid signals in a hydroperoxide-treated protein and in a sacroplasmic protein. A. Rockfish myofibrillar protein upon removal from the freeze-drier (lower trace). The same material 40 min after incorporating a small amount of a hydroperoxide mixture prepared from a marine oil (upper trace). B. Freeze-dried rockfish sacroplasmic protein after storage in air at room temperature for 1 day. Arrows denote g = 2.

observed for dilute solutions of low molecular weight radicals. Without exception, all powdered materials of proteinaceous nature, which exhibited any type of signal at all, gave a single absorption line in the "free-spin" or g = 2 region. The g = 2 signal is exemplified in Figure 1a (for solvent-extracted myofibrillar protein), or in Figure 1b for human serum albumin. I will have more to say about the g = 2 signal; however, let us concern ourselves for the moment with other resonances which are seen in those samples containing oxidizable lipid in addition to protein.

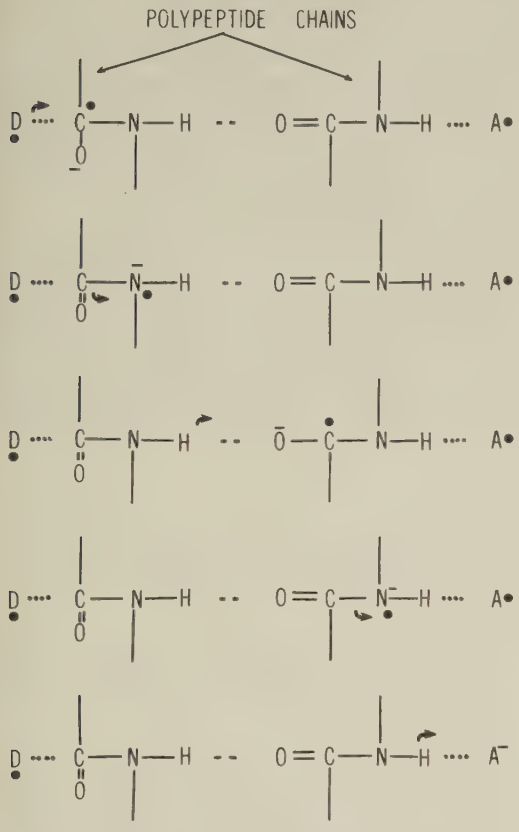
Freeze-dried Pacific cod, silver salmon, rockfish, and other marine fish, though devoid of the g = 2 signal initially (before lipid oxidation has taken place) soon give rise to two resonances when samples are exposed to air—the central g = 2 resonance and, downfield (to the left) from the central resonance, an area of EPR activity which I have designated as the "lipid signal" region (Figures 2 and 3). Unlike tissue samples, many single proteins considered to be quite pure exhibit a g = 2 resonance only. When, however, a thin film of oxidizable lipid is deposited on such materials and the mixture is exposed to air, in addition to the central resonance, a lipid signal is also observed (Figure 1). A preliminary study of lipid signals in various models is to be found in the recent work of the author (1970). Although it has not been possible to measure the g-value with the necessary precision needed to fingerprint the radical completely, the available data suggest a radical of the peroxy type. This is further illustrated by the two traces of Figure 3a. No indications of hyperfine splitting (hfs) are also consistent with a radical of this nature.

CHARGE TRANSFER IN TISSUES

In the present study, it is of particular interest to find that in carefully handled freeze-dried tissue samples, there often occurs after the lipid signal reaches a maximum, an abrupt increase in the g = 2 region. In those "native" samples containing a complement of cellular lipid, this may indicate a charge migration (a strong D→A—donor-to-acceptor interaction; strong charge-transfer process) between a cellular constituent acting as a donor and a peroxy radical acceptor. Such a process is also consistent with the observation that it is at this point in time that the lipid signal begins to decay. This draws our attention to the likelihood that once radical content has increased to some critical concentration, overlap of wave functions between a radical acceptor and a donor is sufficient to allow reactions to proceed. The abrupt change in the g = 2 region is illustrated by the spectra of Figure 3. Figure 3b for sacroplasmic protein under air for 1 day is to be compared with the lower trace of Figure 3a for the same material immediately on removal from the freeze-dryer.

Another point in favor of a mechanism of this type is the fact that only proteins are really effective as matrices for the formation as well as for the decay of radicals. Powdered glass, quartz wool, and amino acids are essentially without effect when used as substrates for thin films of reactants. Although there are many unanswered questions concerning the mechanism

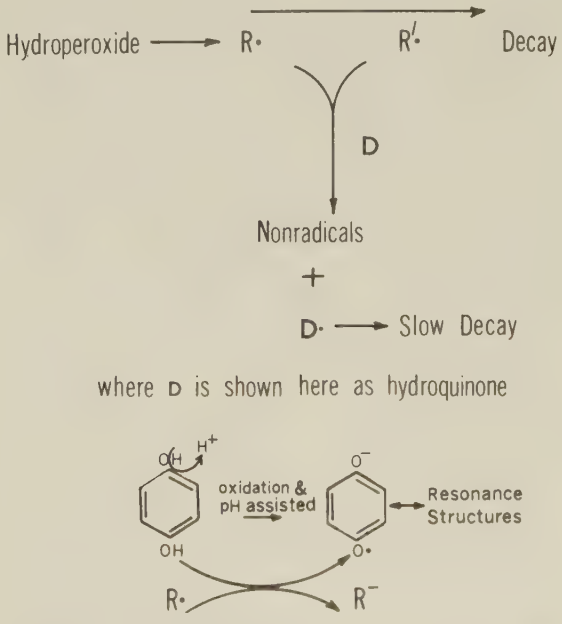
of trapping and charge migration, the data are consistent with the scheme shown below:



SCHEME 1.—Charge-transfer between donor (D) and a free radical acceptor (A) in biological systems. Although the actual pathway is not known with certainty, available data are in accord with the idea that hydrogen bonding of the type shown may play a role in the transfer of charge.

where D is a cellular electron donor material acting in the role of an antioxidant and A is a peroxy radical acceptor. To completely substantiate that such a mechanism does exist would be very exciting indeed for this would be the first instance in which the role of an antioxidant at the molecular level could be designated as a strong D→A interaction.

The data are also in accord with the idea put forth sometime ago by some Russian investigators, that the g = 2 signal in tissue is semiquinone in nature (Chetverikov, Blyumenfel'd, and Fomin, 1965). In the present work, for instance, when hydroquinone or various hydroquinone derivatives with free hydroxyl groups are incorporated into proteins coated with thin films of unsaturated lipid, an enhanced central resonance is obtained which is identical to that obtained for oxidizing tissue alone. For the protein-lipid hydroquinone systems, the author has shown that central resonance consists chiefly of trapped semiquinone radical ions. The g = 2 resonance in "lipid-free" proteins may very well indicate prior lipid oxidation but now at a point in time at which lipid signal has decayed. The g = 2 retention could then be explained because of resonance stability or other stabilizing factors for this species. For instance, some commercial preparations of bovine serum albumin exhibit an EPR signal somewhat displaced from the usual g = 2 signal while others do not. Likewise some of the samples that are EPR-active exhibit fluorescence quite characteristic of malonaldehyde-amino acid interaction (formation of iminopropene derivatives). The various data taken collectively suggest the following mechanism:



SCHEME 2.—Interaction between radicals (R·, R·') and cellular antioxidants (D).

Other radicals, however, may make some small contribution to the central resonance pattern. A recent investigation by Wekell and Roubal[2] has shown that free radicals arise during carbonyl amine browning. What is more, although hfs is seen in early stages of the browning, the signal changes to a single line in the $g = 2$ region as polymerization progresses. The browning reaction, without the implication of free radicals in lipid-protein systems, though not a dominant pathway to pigments, was first discussed by Venolia and Tappel (1958) as a possible cause of color formation during the oxidation of such systems [but because the more recent work, polymeric masses are, for the most part, attributed to lipid peroxy induced protein-protein polymerization together with malonaldehyde cross-linked proteins (conjugated Schiff base)].

Thus, although recent studies in this laboratory have uncovered new facts concerning radicals in lipid-protein systems, the exact nature of the resonances observed in freeze-dried tissue and in dry model mixtures remains to be fully characterized. Nonetheless, this pioneering piece of research has paved the way for use of EPR studies in systems of oxidizing lipids together with other cellular constituents. Concurrent studies have shown that transition metal ion impurities, if present, play only a minor role in radical production. Other studies of this laboratory have shown that protein-free fish bone does not give EPR signals. Freshly prepared freeze-dried tissue samples give no lipid signal resonances, but signal amplitudes grow on exposure to oxygen. Depending on the type of protein, type and amount of lipid, or added material, lipid signals exhibit various lifetimes ranging from hours to years. (For instance, compare Figure 1c for BSA with Figure 2b for 2-year-old FPC.) Polysaccharides are only partially effective as radical matrices.

For living systems, or for any system containing residual and unprotected oxidizable lipid, the implications of the various interactions discussed are significant. It is known that products of lipid oxidation in lipid-protein systems interact with proteins, enzymes, and nucleotides. Not only are these native biopolymers further polymerized by such interactions, constituent building blocks are destroyed (Roubal and Tappel, 1966b, 1967); notable are the sulfur amino acids which have been shown to be easily destroyed by free radicals (Roubal and Tappel, 1966a). In this presentation I have not discussed consequences of unwanted lipid peroxidation in nutritional deficiency states or in other pathologies in living systems. Nevertheless, such lipid-protein interaction is quite significant. The geronotological implications of these reactions leading to the formation of age pigments, based on studies of Roubal and Tappel (1966b) and others, have been reviewed by Bjorksten (1965), Packer, Deamer, and Heath (1967), and Tappel (1968).

[2] Unpublished data; to be published.

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THE RELATION BETWEEN EXERCISE AND BIOCHEMICAL CHANGES IN RED AND WHITE MUSCLE AND LIVER IN THE JACK MACKEREL, *Trachurus symmetricus*

AUSTIN W. PRITCHARD,[1] JOHN R. HUNTER,[2] AND REUBEN LASKER[2]

ABSTRACT

Glycogen, lactic acid, and fat concentration in red and white muscle and glycogen in the liver of jack mackerel, *Trachurus symmetricus*, were measured after periods of forced swimming by *Trachurus* at speeds above, below, and at the sustained speed threshold. Failure to swim at any speed was associated with an almost complete depletion of glycogen in the white muscle only. The trend of glycogen use in the red muscle closely followed that of the liver and was not correlated with failure to swim. Reduction of glycogen levels in red muscle and liver were associated with extended periods of swim- Lipid use was slow and not correlated with fatigured muscle and was insignificant in white muscle. ming. High lipid content was characteristic of e. A decline in lipid concentration after exercise occurred only in the red muscle and only after a swimming period of 6 hr at a subthreshold speed. High lactate levels were characteristic of both muscle types and did not appear to be related to fatigue at any swimming speed.

The high lactate levels in white muscle, the almost complete depletion of glycogen in the white muscle of exhausted fish, and the parallel pattern of glycogen depletion in red muscle and liver suggested that white muscle was the primary locomotor organ near and above the threshold for sustained speed. At these speeds red muscle like the liver may provide nutrients to the white muscle, provided time for mobilization is sufficient. At speeds below the sustained speed threshold our analysis indicated that both the red and white muscle systems were used but the relative significance of the locomotory role played by each system could not be evaluated.

The lateral musculature of many fishes may be readily segregated by color into red and white portions. Typically in active fishes the red muscle makes up from 10 to 20% of the total musculature and is arranged in a thin lateral sheet just beneath the skin whereas the white muscle makes up the underlying mass of the myotome. The two muscle types also differ in the diameter of their muscle fibers, speed of contraction, blood supply, mitochondrial content, patterns of innervation, and glycogen and fat content (Bone, 1966).

The accepted view of the function of red and white muscle tissues in fishes was outlined by Bone (1966). He concluded from his own work on dogfish and from an extensive literature review that the two muscle fibers represent two separate motor systems which operate independently, utilize different metabolites, and serve different locomotory functions, viz., the red muscle is used for slow cruising speeds and functions by aerobic metabolism of fat whereas the white muscle is used for rapid bursts of swimming and is driven by anaerobic glycolysis. Bone's conclusions have subsequently been supported by measurements of oxygen uptake in red and white muscle by Gordon (1968) and by electrophysiological studies on oceanic skipjack, *Katsuwonus pelamis*, by Rayner and Keenan (1967). On the other hand, Braekkan (1956) and Wittenberger (1967) believe the red muscle has no independent locomotor role and functions as a metabolic organ for the white muscle. Electrode recordings from the red muscle (Bone, 1966; Rayner and Keenan, 1967) have provided irrefutable evidence for an independent locomotor function of red muscle at certain slow speeds, but the metabolic independence of the two muscle systems and their metabolic and locomotor function at higher speeds is still open

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to question. Although the roles assigned to the two muscle systems are dependent on swimming speed, no studies have been made on the function of the muscle systems using normally swimming intact animals at known speeds. The objective of this study was to re-examine the metabolic and locomotor roles of red and white muscle by measurement of glycogen, lactate, and fat levels in the muscle and glycogen levels in the liver in fish exposed to various velocity treatments of known strength and duration. Juvenile jack mackerel, *Trachurus symmetricus,* were used in this study because the maximum sustained speed threshold for 6 hr of continuous swimming had already been established for this species (Hunter, 1971), and consequently we were able to relate all of our chemical measurements to known levels of swimming performance.

METHODS AND PROCEDURES

SWIMMING TESTS

Jack mackerel were maintained at a regulated seawater temperature of 18.5° C in a plastic swimming pool 4.57 m diameter and fed an abundant ration of brine shrimp, *Artemia,* and chopped fish and squid each day. The fish were not fed for 20 hr prior to testing. Jack mackerel were tested in an activity chamber patterned after that of Beamish (1968) and described in detail by Hunter and Zweifel (1971). The swimming compartment of the apparatus consisted of a tube 230 cm long and 41 cm in diameter through which seawater could be moved at speeds ranging from 12 to 212 cm/sec. Fish were placed in the tube and forced to swim at a water speed for certain periods varying from 8 min to 6 hr. At the end of the swimming period they were removed and dropped immediately into liquid nitrogen and the frozen fish were stored at —30° C until used for chemical analysis. The time required for removal and freezing did not exceed 1 min.

Speed treatments for the experiments were chosen relative to the 50% endurance threshold for jack mackerel at 22 $L^{0.6}$/sec for 6 hr of swimming where L is total length (Hunter, 1971). Five jack mackerel, mean length 14.6 cm, were tested at the subthreshold speed of 19.6 $L^{0.6}$/sec (98 cm/sec); 14 jack mackerel, mean length 16.3 cm, were tested at the near threshold speed of 21.1 $L^{0.6}$/sec (113 cm/sec); and 10 jack mackerel, mean length 14.7 cm, were tested at the superthreshold speed of 27.7 $L^{0.6}$/sec (139 cm/sec). Fish tested at the subthreshold speed swam continuously for 6 hr and were sampled at the end of that period. Fish tested at the threshold speed were divided into two groups: seven fish that were sampled after 6 hr of continuous swimming; and seven fish that fell from exhaustion at some time during the 6-hr period. The latter group of seven fish were quickly removed from the apparatus and frozen as soon as they fell against the rear screen. Fish tested at the superthreshold speed were also divided into two groups: those that swam successfully for 8 min; and those that failed after 8 or less minutes of swimming.

Ten jack mackerel, mean length 14.5 cm, were used as controls. Five of the control animals were removed from the holding tank, placed in the apparatus, allowed to swim for 30 min at the slow speed of 6.2 $L^{0.6}$/sec (30 cm/sec), removed, and frozen. The other five control fish were removed from the holding tank and immediately frozen. The data from these two control groups were later combined because no difference between them was detected.

CHEMICAL ANALYSES

White and red muscle were dissected from the frozen fish while still frozen. One lateral strip of red muscle was used for fat analysis and the other divided into two equal portions for lactate and glycogen analysis respectively. About 1 g of white muscle from the dorsal portion of the myotome was used for glycogen determinations, 0.5 g for lactate, and 0.5 g for fat measurements. Fish were returned to the freezer and liver samples (0.1-0.2 g) were analyzed for glycogen about a month after the muscle determinations.

For lactate measurements muscle was quickly cut into small pieces, weighed, and homogenized in 10% trichloroacetic acid in prechilled tubes. Proteins and cellular debris were spun down in a clinical centrifuge. Aliquots of the protein-free supernatant fluid were analyzed for lactate enzymatically using the test reagents supplied by SIGMA Chemical Company.[3] The test is based on the conversion of nicotine adenine nucleotide (NAD) to the reduced form (NADH) as lactate is converted to pyruvate by lactate dehydrogenase. All readings were made at 340 mμ on a Beckman DU spectrophotometer. Results are expressed as mg of lactic acid per 100 g wet weight muscle tissue.

Muscle and liver samples for glycogen determinations were dropped into preweighed graduated centrifuge tubes containing 3 ml of 30% potassium hydroxide. Glycogen was precipitated with alcohol and determined according to the method of Montgomery (1957). All readings were made at 490 mμ on a Beckman DU spectrophotometer. Results are expressed as mg glycogen (as glucose) per 100 g wet weight in the case of muscle, and as percent glycogen in the case of liver.

Muscle tissue was dried in an oven at 60° C to constant weight for fat analysis. Fat was removed by a soxhlet extraction with chloroform-methanol (2:1, v:v). After the extraction the solvent in the tissue was evaporated and the difference in weight of the tissue recorded (Krvarić and Mužinić, 1950).

[3] P.O. Box 14508, St. Louis, Mo. 63178. Reference to commercial products does not imply endorsement.

RESULTS

Fish that swam continuously for 6 hr at the subthreshold speed of 98 cm/sec and at the threshold speed showed no difference in the glycogen content of the white muscle from the controls (Table 1). On the other hand, in fish

TABLE 1.—Glycogen in red and white muscle, and liver of jack mackerel following various forced swimming conditions. Red and white muscle glycogen in mg per 100 g wet weight; liver glycogen is percent of wet weight. -- indicates measurement was lost during analysis.

	Controls			19 $L^{0.6}$ Subthreshold speed			21 $L^{0.6}$ Threshold speed successes		
	Red	White	Liver	Red	White	Liver	Red	White	Liver
	76.6	85.9	6.42	15.23	53.90	0.125	26.71	204.3	0.043
	102.8	--	9.49	52.80	143.2	4.36	33.33	159.4	1.63
	176.3	--	22.74	192.5	76.69	.317	37.59	80.74	1.85
	277.8	276.8	8.75	145.2	316.2	8.31	95.93	492.8	.125
	562.0	142.6	18.59	147.6	102.6	3.16	152.4	223.2	3.42
	706.0	157.9	18.17				191.7	638.0	3.19
	1075	267.8	10.26				475.1	216.3	1.38
	1394	72.9	17.00						
	1417	71.1	11.82						
	1706	216.5	24.24						
Mean	749.4	161.4	14.75	[1]110.7	138.5	[1]3.25	[1]144.7	287.8	[1]1.66

	21 $L^{0.6}$ Threshold speed fatigued			28 $L^{0.6}$ Superthreshold speed-individually fatigued			28 $L^{0.6}$ Superthreshold speed, 8-min test		
	Red	White	Liver	Red	White	Liver	Red	White	Liver
	11.5	26.50	.078	--	--	0.546	--	39.93	10.00
	16.00	4.51	.034	149.6	8.02	4.73	298.6	141.9	10.00
	17.10	20.63	3.30	215.0	19.56	3.83	473.3	25.44	3.16
	40.55	27.77	5.04	490.4	6.76	13.23	533.1	141.0	10.94
	104.8	--	11.96	654.5	18.49	5.81	553.9	57.22	11.31
	151.8	11.15	.820						
	241.5	50.2	12.50						
Mean	[1]83.27	[1]23.46	[1]4.82	377.4	[1]13.21	[1]5.63	464.7	[1]81.1	9.08

[1] Differed from the controls, $P \leq 0.05$, Mann Whitney U test (Siegel, 1956).

that failed to swim the full 6 hr at the same speed the glycogen levels in the white muscle were lower and were different from the controls ($P = 0.001$ Mann Whitney U test, Siegel, 1956). Glycogen levels in white muscle of all fish tested at the superthreshold velocity were also much lower and statistically different from the controls ($P = 0.05$). The lowest glycogen levels of all were in fish that failed from exhaustion at superthreshold speeds. The values in these exhausted fish were statistically different from those of fish that swam at the same speed but which were removed after 8 min of swimming before they could fall from exhaustion. In sum, strenuous exercise and exhaustion regardless of speed were associated with a marked depletion of glycogen reserves in the white muscle, whereas successful swimming for 6 hr at subthreshold or threshold speed produced no. significant change in white muscle glycogen.

The glycogen content of the liver and red muscle were lower and different from the controls in fish tested at threshold and subthreshold speeds ($P = 0.05$). At superthreshold speed, on the other hand, the glycogen content of the red muscle was not different from the controls and that of the liver was different only in fish that failed from exhaustion ($P = 0.02$).

Thus, the trends in the levels of red muscle and liver glycogen in relation to swimming speed were nearly the reverse of that for white muscle glycogen. Low levels of glycogen in red muscle and liver were associated with slow speeds that could be sustained for extended periods. These results suggest that glycogen from red muscle and liver provide energy to the white muscle at nearly all swimming speeds. We believe that no drop occurred in red muscle glycogen in fish fatigued at high speeds because the time was too short for the white muscle to mobilize significant amounts of glycogen. This view is supported by the negative correlation between the level of glycogen in the red muscle and swimming time to fatigue at threshold speed. This is illustrated in the following table:

Threshold speed $= 21\ L^{0.6}$

Time to fatigue (*min*)	Glycogen in red muscle (*mg per 100 g wet weight*)
282	11.5
110	16.0
131	17.1
79	40.6
15	104.
38	241.

($r_s = -0.857$, $P < 0.05$)

In fish exercised at the superthreshold speed the lactic acid content of the red and white muscle was considerably above that of the controls and statistically different from them ($P = 0.05$) (Table 2). At threshold and subthreshold speeds, the lactic acid concentration in red and white muscle formed no distinct pattern. At threshold speed the lactate levels of red and white muscle were about the same as the controls and did not differ from them except for one case where the values were actually lower

TABLE 2.—Concentration of lactic acid in red and white muscle of jack mackerel following various forced swimming conditions. Values given are mg lactic acid per 100 g wet weight.

	Controls		19 $L^{0.6}$ Subthreshold speed		21 $L^{0.6}$ Threshold speed successes	
	Red	White	Red	White	Red	White
	40.49	233.0	94.6	520.9	20.60	310.4
	59.29	425.3	97.6	596.6	22.83	344.5
	71.58	387.5	99.2	521.9	26.79	385.3
	77.46	521.0	117.1	630.3	45.66	464.8
	79.53	570.3	156.8	762.3	56.94	403.1
	82.19	341.5			82.19	345.2
	86.76	390.6			83.87	410.2
	86.76	319.0				
	86.76	553.4				
	95.44	589.6				
Mean	76.63	433.1	[1]113.1	606.4	[1]48.41	380.5

	21 $L^{0.6}$ Threshold speed fatigued		28 $L^{0.6}$ Superthreshold speed - individually fatigued		28 $L^{0.6}$ Superthreshold speed, 8-min test	
	Red	White	Red	White	Red	White
	26.23	564.6	101.2	422.9	124.2	724.1
	39.61	545.3	108.4	538.6	132.3	668.9
	56.58	404.4	120.4	723.1	189.0	799.9
	66.6	499.2	151.9	745.8	202.8	807.4
	86.76	489.7	230.5	800.6	237.4	733.5
	122.5	486.2				
	205.5	646.1				
Mean	86.25	519.4	[1]142.5	[1]646.2	[1]177.1	[1]746.8

[1] Differed from the controls, $P \leq 0.05$, Mann Whitney U test (Siegel, 1956).

than the controls; at this subthreshold speed lactate levels of red and white muscle were higher than the controls and differed statistically ($P = 0.02$). We have no explanation for these differences except to suggest that the high muscle lactate concentration in the control animals may have obscured changes resulting from moderate exercise. A larger sample size may be required to obtain reliable measurements of differences in lactic acid concentration caused by moderate exercise.

Muscle lactate level did not appear to be related to fatigue at any swimming speed. Lactate levels in fish that fatigued at the threshold speed were not different from the controls. Fish that failed at superthreshold speeds had a higher muscle lactate level than did the controls but the level did not differ from that of fish that swam at the same speed but were removed before they became exhausted. These results suggest that high lactic acid concentration in muscle was not the principal cause of exhaustion.

Red muscle contained considerably more fat per unit weight than white muscle. Indeed, white muscle fat levels were almost undetectable in many cases (Table 3). White muscle fat levels did not differ from the control at any speed level. Red muscle fat did not differ from the controls at threshold and superthreshold speeds but at the subthreshold speed the mean level of fat in the red muscle was lower than the controls and differed statistically from them ($P = 0.02$). Thus only when the fish swam for at least 6 hr at subthreshold speed was there evidence of fat utilization in the red muscle.[4] The reduction in fat in the red muscle suggests that the red muscle system may have been used at the subthreshold velocity. On the other hand, presence of high muscle lactate in both red and white muscle and the drop in red muscle and liver glycogen at subthreshold speeds implies that the white muscle was also active.

[4] In an earlier and preliminary experiment, five smaller jack mackerel, mean length 9.2 cm, swam at the subthreshold speed of 12.7 $L^{0.6}$/sec (48 cm/sec) for 18 hr without failure and we recorded a decrease in the mean fat content of red muscle from 23.7% (range, 20.4-28.4%; $n = 5$) to 18.0% (range, 16.2-20.8%; $n = 5$) ($P<0.05$).

TABLE 3.—Fat analyses in red and white muscle of jack mackerel following various forced swimming conditions. Where 0.0% is given for white muscle, only traces of fat were found with the chloroform-methanol extraction. For convenience zeros were used for averaging. Values given as percent dry weight of tissue.

	Controls		19 $L^{0.6}$ Subthreshold speed		21 $L^{0.6}$ Threshold speed successes	
	Red	White	Red	White	Red	White
	20.16	1.98	15.07	0.230	16.91	0.0
	21.00	0.0	15.69	2.12	22.45	0.0
	21.78	.337	16.19	1.24	23.80	2.19
	22.24	1.15	20.54	0.0	24.67	0.0
	23.05	1.32	24.63	0.0	24.97	1.54
	25.11	0.0			26.43	4.09
	25.14	.390			29.37	4.10
	25.89	.924				
	27.29	2.65				
	32.32	2.20				
Mean	24.40	1.10	[1]18.42	.718	24.08	1.70

	21 $L^{0.6}$ Threshold speed fatigued		28 $L^{0.6}$ Superthreshold speed - individually fatigued		28 $L^{0.6}$ Superthreshold speed, 8-min test	
	Red	White	Red	White	Red	White
	21.30	0.0	16.54	0.0	21.96	2.01
	21.97	0.0	16.71	0.0	23.08	.04
	22.47	0.0	26.47	1.22	24.20	.18
	28.70	0.0	28.19	.732	25.57	.50
	30.11	2.56	28.70	2.13	29.54	2.26
	32.08	1.12				
	32.90	6.46				
Mean	27.08	1.45	23.32	.816	24.87	.998

[1] Differed from the controls, $P = 0.02$, Mann Whitney U test (Siegel, 1956).

DISCUSSION

Control levels of jack mackerel white muscle glycogen were similar to those recorded by Canadian workers for mixed red and white muscle in salmonids (Black, Robertson, and Parker, 1961; Black et al., 1962; Connor et al., 1964) and to those from a variety of marine teleosts (Beamish, 1968; Fraser et al., 1966; Wittenberger, 1968; Wittenberger et al., 1969). Red muscle glycogen has not often been separately determined. Our mean control value of 750 mg percent was somewhat higher than the mean of 420 mg percent reported by Wittenberger (1968) for *Trachurus mediterraneus ponticus*, a related species from the Black Sea. Fraser et al. (1966) gave a range of 215 to 279 mg percent for red muscle glycogen of cod, based on analysis of three fish in a relaxed (anesthesized) state. Wittenberger et al. (1969) reported 320

mg percent in a clupeid, *Harengula humeralis*. A much higher level of 1866 mg percent was given by Bone (1966) for dogfish. In most cases, the concentration of glycogen in red muscle was considerably higher than in white muscle.

Liver glycogen controls in jack mackerel were much higher than those reported previously in teleosts. Connor et al. (1964) for example, obtained values of about 1% in chinook and sockeye salmon and steelhead trout, and found that moderate exercise associated with ascending fishways had no effect on liver glycogen levels. Black et al. (1960) reported liver glycogen levels of 0.5-4% in rainbow trout, and Dean and Goodnight (1964) obtained 0.8-3% in four species of warmwater centrachid fishes. Values similar to ours were reported by Wittenberger and Diaciuc (1965) in carp (13.8%) and by Bellamy (1968) in recently fed red piranha (10.3%). Even if a high degree of gluconeogenesis were operative in jack mackerel, it seems unlikely that this could entirely explain the high levels of liver glycogen.

Control levels of glycogen in jack mackerel white muscle appeared to be similar to those in other fishes. However, in the red muscle and especially in the liver, glycogen levels were usually higher than in fishes studied earlier.

The most striking finding of this study was the virtually complete depletion of glycogen in the white muscle of fish that failed from exhaustion. The depletion of glycogen in white muscle occurred in all fish that failed regardless of the speed of swimming or how long they swam. In fish that did not fail at a near threshold speed of 21 $L^{0.6}$/sec (Hunter 1971) the glycogen in the white muscle did not differ from controls, whereas in the fish that failed, glycogen in the white muscle was at nearly the same low level as it was in fish that failed after a few minutes of exertion at a much higher speed. Red muscle glycogen was also depleted at some swimming speeds but the pattern of glycogen depletion in red muscle closely paralleled that of the liver. Red muscle had one-fifth the lactate found in white muscle on a percent basis but only about one-fiftieth on an absolute basis because the mass of white muscle exceeds the red by 10 to 1.

The high lactate levels in the white muscle, the almost complete depletion of glycogen in the white muscle of exhausted fish, and the parallel pattern of glycogen depletion in red muscle and liver all point to the same hypothesis. In jack mackerel at threshold and higher speeds the energy used for swimming was derived primarily from glycolysis in the white muscle which was the principal locomotor organ. Red muscle like the liver may serve as a storage organ whose resources could be used to drive the white muscle, given sufficient time for mobilization. Thus at threshold speeds, red muscle function appeared to be tied to that of the white and it could not be considered as acting independently. No change in red muscle glycogen was detected at the highest test speed, possibly because time was insufficient to mobilize the glycogen reserves other than in the white muscle itself. This time dependency for mobilizing red muscle glycogen under conditions of strenuous exercise could explain why Bone (1966), Wittenberger and Diacuic (1965), Wittenberger (1968), and Fraser et al. (1966) detected no change in red muscle glycogen after strenuous exercise. It must be remembered that in all of these previous studies the strength and the duration of the exercise was unknown, except that it was considered to be extreme.

The decrease in fat content plus the high lactate levels suggest that the red muscle was used for swimming at subthreshold speeds. Bilinski (1969) showed that the rate of oxidation of fatty acids in red muscle of rainbow trout and sockeye salmon exceeded that in the white muscle by one or more orders of magnitude depending on the fatty acid substrate. On the other hand, neither the high oxidative capacity nor the decline in lipid levels in red muscle with moderate exercise are sufficient evidence for an independent locomotor role. In addition, the presence of high lactate levels in white muscle and the drop in the glycogen content of the white muscle indicated that the white muscle was also used at the subthreshold speed of 19 $L^{0.6}$/sec. The electrophysiological evidence for indepen-

dent locomotor activity of the red muscle cannot be ignored. At some speed slower than any used in the present experiment jack mackerel may depend only on red muscle for propulsion and on lipids for fuel. At what velocity red muscle begins to play a major role or how significant this speed may be in the life of the animal are questions that remain to be answered. The most tenable explanation for these data is that both muscle systems were used at the subthreshold speed but we are unable to choose which system played the more significant role.

Jack mackerel appear to be specialized in body form and swimming capabilities for high-speed continuous swimming (Hunter, 1971). Thus the physiological characteristics we have described, namely use of glycolysis in white muscle for swimming, high liver glycogen levels, and tolerance of high muscle lactate levels may represent specializations for high-speed swimming and may not be representative of the general pattern in fishes. On the other hand, *Trachurus* may share these characteristics with other fishes of similar habits, for example other carangids and the scombroid fishes. It seems possible that evolution may have favored the development of these physiological characteristics because severe velocity limits may be set by aerobic lipid metabolism.

ACKNOWLEDGMENTS

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Sebastes variegatus, SP. N. FROM THE NORTHEASTERN PACIFIC OCEAN (PISCES, SCORPAENIDAE)

JAY C. QUAST[1]

ABSTRACT

A new scorpaenid fish, *Sebastes variegatus*, from the Gulf of Alaska is characterized by an elongate body that tapers symmetrically anteriorly and posteriorly; presence of preocular, postocular, tympanic, and parietal spines and lack of supraocular, coronal, and (usually) nuchal spines; 18 (rarely 17 or 19) rays in the pectoral fin; a second anal fin spine that is longer than the third; black membranes in the spinous dorsal and caudal fins; a dark brown to jet black peritoneum; and a dark blotched pattern on the sides that is interrupted over the posterior 2/3 of the body by an unpigmented band along the lateral line. The known geographic range is from Unimak Pass (Aleutian Islands) to Queen Charlotte Sound (British Columbia).

On February 28, 1967, the Bureau of Commercial Fisheries RV *Murre II* obtained three specimens of a new species of rockfish from the vicinity of Point McCartney in Frederick Sound, southeastern Alaska. The specimens were captured when a 6-ft-diameter Isaacs-Kidd trawl inadvertently was allowed to touch bottom at 135 m.

The specimens were tentatively identified as *Sebastes zacentrus* but were not adequately described by Phillips' (1957) account of this species. In some respects they seemed to be intermediate between *S. zacentrus* and *S. proriger*. The evidence for an undescribed species became convincing when additional specimens were taken, and the similarity to *S. zacentrus* and *S. proriger* was underscored when several more specimens were found in old collections that had been labeled as these species.

The species is placed in *Sebastes* Cuvier in concurrence with opinions of numerous authors that northeastern Pacific *Sebastodes* are congeneric with North Atlantic *Sebastes*—including Matsubara (1943b:178); Tsuyuki, Roberts, Lowes, Hadaway, and Westrheim (1968:2494); Eschmeyer (1969:104); Chen (1969:12); and American Fisheries Society (personal communication with Reeve M. Bailey, University of Michigan, December 1969).

The name *variegatus* refers to the contrasting coloration of most specimens when fresh. According to Brown (1956:830), the word means "of different sorts, particularly colors." I suggest "harlequin rockfish" as the common name.

MATERIALS AND METHODS

The study utilized 39 specimens of *S. variegatus* and included 35 from the ichthyological collection of the National Marine Fisheries Service Biological Laboratory at Auke Bay, Alaska, and 3 from the collection at the Institute of Animal Resource Ecology, University of British Columbia, Vancouver, Canada. This series included 21 males, 13 females, and 4 of unknown sex, and no important differences were found between the sexes in measurements, counts, or coloration. Measurements on the University of British Columbia specimens were not as complete as those from the Auke Bay Laboratory; hence the numerical basis of the morphometric analysis varies by 3, depending on the character. An additional male specimen was used for electrophoretic analysis.

Methods for counts and measurements are based on Hubbs and Lagler (1949:8-15), with the following exceptions or modifications. In counts: tubed lateral line scales on caudal fin lie after hypurals; tubed scales over hypurals are included in count of tubed lateral line scales

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on body; and scale rows are taken below lateral line. In measurements: standard length (SL) is measured from anterior upper lip or upper teeth, whichever is more anterior, to caudal base; head length is measured from midline on upper jaw to limit of opercular flap; interorbital width equals bony interorbital measurement; lower jaw projection equals distance that lower jaw extends anteriorly to upper jaw, projected on longitudinal axis of fish; length of gill raker equals length of raker at bend of gill arch, measured from fleshy tip to, but not including, T-shaped base; length of prenarial pore equals length of enlarged pore on anterior snout near junction of premaxilla and premaxillary process; length of subnarial pore equals length of large pore on a shelf formed by upper margin of lacrimal bone, immediately below nares; body depth (pelvic) is measured from articulation of pelvic fin spine; body depth (anal) is measured from base of anal fin anterior to first anal spine; length of pectoral fin is measured from base of uppermost ray to apex of fin when fin is aligned with longitudinal axis of fish; length of soft dorsal fin base is measured from axil of last dorsal fin spine to axil of last soft ray; length of base of anal fin is measured to axil of last ray; and length of pelvic fin is measured from articulation of pelvic fin spine to tip of fin.

Terminology for spines and ridges of head follows Jordan and Evermann (1898, II:1765, 1768).

Sebastes variegatus SP. N.

(FIG. 1-3)

HOLOTYPE

(Original number AB[2] 69-25); 201 mm SL; male; Gulf of Alaska south of Kodiak Island (approximately long 152°25′ W and lat 56° 25′ N); on bottom in 284 m; 12 June 1969; Ted Shigyo, U.S. fishery observer on Japanese stern trawler *Kirishima Maru.* California Academy of Sciences, San Francisco, Calif. (Number CAS 24857).

[2] Museum designations: AB—National Marine Fisheries Service Biological Laboratory, P.O. Box 155, Auke Bay, Alaska 99821; BC—University of British Columbia Institute of Animal Resource Ecology, Vancouver 8, British Columbia, Canada; CAS—California Academy of Sciences, Golden Gate Park, San Francisco, Calif. 94118; SIO—University of California, San Diego, Scripps Institution of Oceanography, P.O. Box 109, La Jolla, Calif. 92037; USNM—U.S. National Museum, Washington, D.C. 20560.

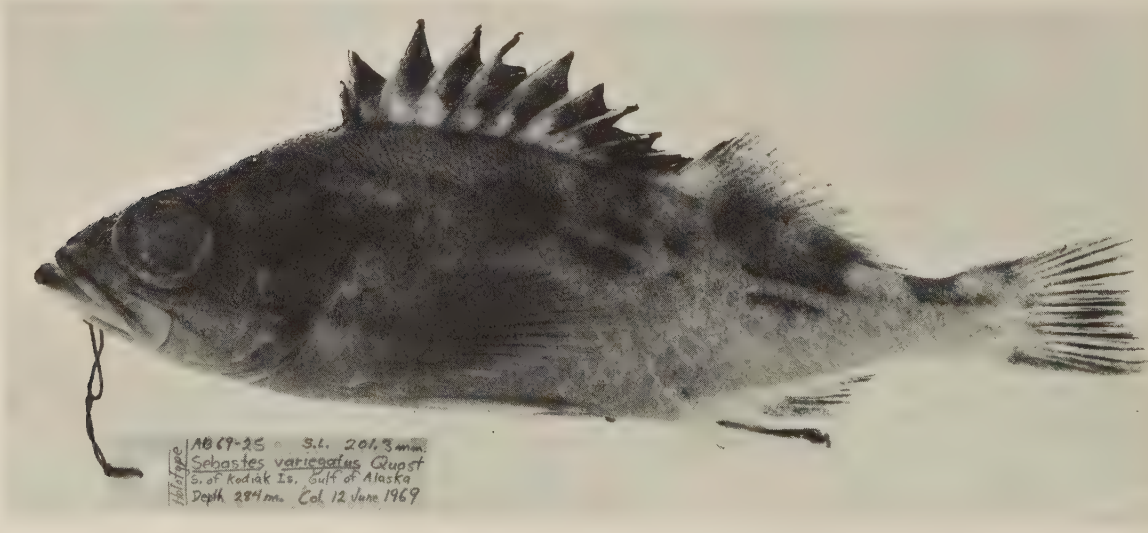

FIGURE 1.—*Sebastes variegatus* sp. n., holotype CAS 24857; 201 mm; male.

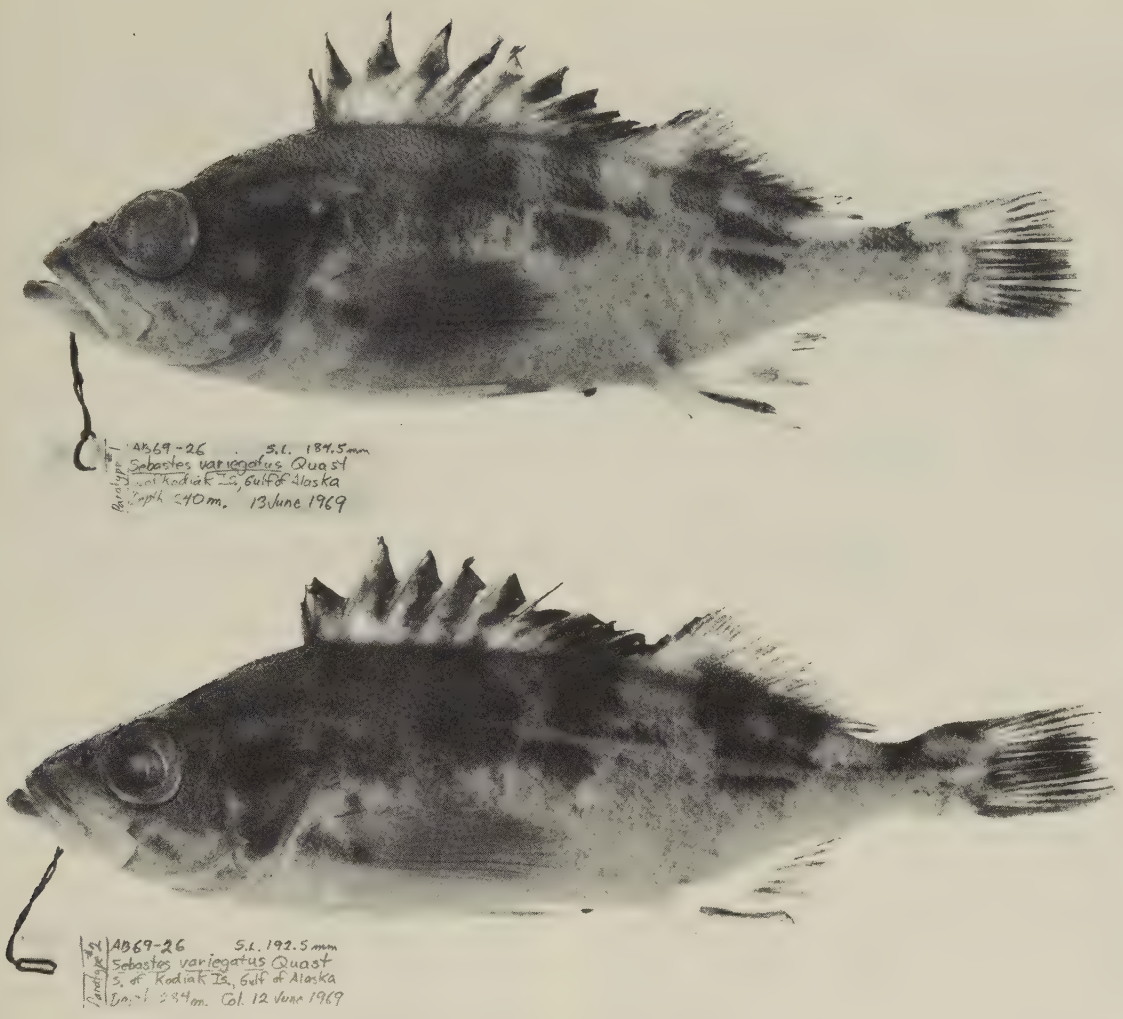

FIGURE 2.—*Sebastes variegatus* sp. n., paratypes USNM 204937 (upper) and SIO 70-171 (lower); 185 and 193 mm; both males. (Depth and date of Paratype 2 should be the same as for Paratype 1.)

PARATYPES (NOS. 1 and 2)

(Original number AB 69-26); U.S. National Museum 204937, 185 mm, and SIO 70-171 University of California, San Diego, 193 mm; males; Gulf of Alaska south of Kodiak Island (approximately long 152°25′ W and lat 56°25′ N); on bottom in 240 m; 13 June 1969; Ted Shigyo, U.S. fishery observer on Japanese stern trawler *Kirishima Maru.*

OTHER MATERIAL

WESTERN GULF OF ALASKA. Davidson Bank: AB 68-598 (3, 175-182 mm SL), 26 October 1968, RV *Miller Freeman.* East of Simeonof Is.: BC 65-70 (2, 145-196 mm SL), 5 August 1964, *G. B. Reed.* Kodiak Island vicinity: AB 67-171 (1, 235 mm SL), 19 April 1964, *Taiyo Maru No. 81*; AB 67-22 (1, 277 mm SL), 21 April 1967, *Yutaka Maru;* AB 66-154 (1, 286

FIGURE 3.—Melanistic specimen of *Sebastes variegatus* (AB 67-62); 277 mm SL; Kodiak Island vicinity.

mm SL), 8 May 1966, *Taiyo Maru No. 2;* AB 67-68 (1, 210 mm SL), 28 April 1967, *Yutaka Maru;* AB 64-847 (1, 153 mm SL), 10 September 1964, *Taiyo Maru No. 77;* AB 68-10 (1, 197 mm SL), 3 September 1964, *Taiyo Maru No. 77;* AB 63-120 (2, 137-143 mm SL), 4 August 1962, RV *Yaquina.* MIDDLE GULF OF ALASKA. Portlock Bank (S of Seward): AB 63-123 (2, 157-230 mm SL), 13 August 1962, RV *Yaquina;* BC 64-292 (1, 223 mm SL), 26 August 1963, *G. B. Reed.* Vicinity of Port Bainbridge (ESE of Seward): AB 64-651 (2, 120-132 mm SL), 16 July 1963, RV *Yaquina.* West of Montague Is.: AB 64-645 (1, 109 mm SL), 16 July 1963, RV *Yaquina.* South of Montague Is.: AB 68-527 (5, 147-169 mm SL), 18 July 1968, RV *Oshoro Maru.* Off Cape Yakataga: AB 68-526 (1, 193 mm SL), 25 July 1968, *Daishin Maru No. 12.* Off Yakutat: AB 70-23 (2, 245-278 mm SL), 15 July 1968, RV *Oshoro Maru.* EASTERN GULF OF ALASKA. Queen Charlotte Sound: BC 70-2 (1, 237 mm SL), 12 June 1970, RV *G. B. Reed* (Electrophaerogram). Frederick Sound: AB 67-11 (3, 241-266 mm SL), 28 February 1967, RV *Murre II.* Forrester Is. vicinity: AB 68-524 (1, 211 mm SL), 24 July 1968, *Ishikari Maru;* AB 68-525 (1, 242 mm SL), 24 July 1968, *Ishikari Maru.* West of Dall Is.: AB 68-24 (3, 146-171 mm SL), 5 November 1956, RV *John N. Cobb.*

DIAGNOSIS

A species of *Sebastes* with body outline slender and symmetrically terete; preocular, postocular, tympannic, and parietal spines present and supraocular, coronal, and (usually) nuchal spines absent; second anal spine longer than third; symphyseal knob not prominent; 18 (rarely 17 or 19) rays in pectoral fin; black spinous dorsal and caudal fin membranes; peritoneum dark brown to jet black; and dark blotched pattern of pigmentation on back and sides, the pattern partly interrupted by a broad unpigmented band that extends over posterior 2/3 of body and includes lateral line.

MORPHOLOGY (PRINCIPALLY FROM HOLOTYPE AND PARATYPES)

Body form, including head, slender, the outline smooth and tapers nearly symmetrically

to body axis anteriorly and posteriorly. Body near head flattened laterally, body width slightly greater than 1/2 body depth at pelvic fins. Interorbital region flat to convex. Cranial ridges over orbit usually lower than dorsal profile of head when viewed from side, but sometimes tangent or project slightly above the profile. Dorsal outline of head usually smooth and slightly concave between snout and nape. Lower jaw projects and enters dorsal profile of head. Symphyseal knob weakly developed or absent. Posterior profile of caudal fin indented; that of anal fin slants upward-posteriorly. Pectoral fin symmetrical; both it and pelvic fin extend to or nearly to vent.

Head spines low but strong and well developed—nasal, preocular, postocular, tympannic, and parietal present. Other cranial spines absent except occasionally one nuchal present. Two opercular spines diverge slightly and are contained within outline of opercular flap. Some specimens with one (rarely two) small spines on lower opercle. Two strong suprascapular spines. Five preopercular spines: upper three longest and diverge slightly; second from top longer than either first or third. Lower two preopercular spines broadly triangular and strong. Lacrimal bone has two blunt or rounded prominences ventrally but no spines. Spines absent on suborbital bones and stay.

Spinous rays in dorsal fin strong and sharp, third to fifth usually longest. Interspinous membranes markedly indented posteriorly in each space; indention varies from about 1/2 of length of following spine in first to about 1/4 of following spine in fourth space and posteriorly. Spinous rays of anal and pelvic fins strong and prominent; second anal spine longest, and 14-23% of its length exceeds third. Each soft ray in dorsal fin branched at least once, usually with posterior and sometimes anterior branchlet divided again. Bordering principal rays on caudal fin simple, but branched rays have three sets of dichotomies each (end in eight branchlets). Soft rays of anal fin branched at least once, and usually each branchlet divided again; sometimes posterior branchlet divided several times. Soft rays of pelvic fin branched at least twice, usually with further dichotomies. Uppermost ray of pectoral fin simple; ventrally, degree of branching increases from one dichotomy to 1-1/2 or 2; lowermost rays slightly thickened and simple, rarely with one dichotomy.

Squamation on head ctenoid and nearly complete—includes snout between premaxillary processes (in holotype but not in paratypes), sides of snout, dorsal surface of head, preopercle, opercle, lacrimal area, maxilla, mandible, and branchiostegals. Lips and branchiostegal membranes not scaled. Body fully scaled; scales finely ctenoid on body sides, but ctenation reduced on belly and breast and even more reduced to absent on scales of isthmus, which are all small. A few small, smooth scales extend in line posteriorly from axilla of each pelvic fin. Scales on bases of vertical fins and between pelvic fins markedly reduced in size. Scaled areas on dorsal fin spines extend nearly to tips. Interspinous membranes usually scaled on proximal 1/3; scaled areas usually extend farther distally near fin spines. Dorsal fin squamation mostly smooth on holotype but mostly ctenoid on paratypes. Scales usually absent in areas of dorsal fin membranes with heavy black pigmentation. Rays of soft dorsal fin scaled nearly to tips, but membranes not as completely scaled. Scaled areas of soft dorsal fin usually limited to proximal 2/3 of fin—all scales ctenoid. Squamation of caudal fin ctenoid, its membranes scaled nearly to end of fin. Posterior unpigmented border of fin naked. Caudal rays scaled only near their bases (but scales possibly lost from more distal portions of rays in specimens of this series owing to collection methods). Squamation of anal fin ctenoid; fin spines scaled nearly to tips; interspinous membranes naked except for narrow area bordering third spine. Membranes between anal soft rays scaled over proximal 1/2-3/4; scaled areas extend farther distally on soft rays. Pelvic fin has spines and soft rays scaled nearly to tips; scales mostly smooth. Upper pectoral rays scaled nearly to tips, but squamation absent at unpigmented tips of rays. Proceeding downward on ventral 1/2 of pectoral fin, rays have progressively more naked area distally; extent of naked area pro-

gresses from about 1/4 length of uppermost single ray to about 1/2 of lowermost ray.

Upper jaw teeth simple and in two modal sizes. A larger outer row, sometimes mainly uniserial, extends nearly length of each premaxillary. Anteriorly, teeth larger yet and band broadens. A medial gap where tooth patches at lower jaw tip meet upper jaw (small papilla in middle of gap). A smaller inner row of teeth, may also be uniserial, in narrow band over most of premaxillary; it loses uniserial character near large anterior teeth, skirts behind them and continues to medial gap. Teeth of lower jaw simple, uniserial, and similar in size to large teeth in row of upper jaw. Anteriorly, lower jaw teeth are larger and in a tuft that is separated from midline by narrow gap—with jaws shut, from 1/2 to all of each tuft is visible. Vomerine teeth small, in broad V-shaped patch, and sometimes slightly enlarged to suggest a tuft at apex of patch. Palatine teeth small, uniserial except anteriorly where row broadens. Gill rakers slender and pointed, one at bend of arch steps 1.6-2.2 (mode 1.8) into orbit. A small slit behind last gill arch.

A large prenarial pore on side of snout in angle formed by premaxillary process and premaxilla. A larger subnarial pore on flat shelf immediately below nares—outer margin of shelf formed by part of dorsal margin of lacrimal bone. Smaller pores occur singly above nares and below nasal spine. Two large pores on ventral surface of lacrimal bone—one slightly anterior to vertical through subnarial pore and another slightly posterior to this vertical and beneath anterior blunt projection on ventral surface of lacrimal bone. Four prominent pores on each mandible—first in or near fold formed by symphyseal knob and remaining three in a line along ventral surface.

Suborbital bones continuous around eye; second (suborbital stay) conforms to Type 1 of Matsubara (1943a:10-13), i.e. stay tapers to point posteriorly and does not reach preopercle. Sensory canal terminates at midlength of stay. Third and fourth suborbitals tubular. Seven branchiostegals, 5½ on ceratohyal and 1½ on epihyal.

COLOR

Background color of body (based on Kodachrome transparencies of seven freshly caught young adult specimens from Davidson Bank, near Unimak Pass, and the Yakutat vicinity) varies from light pink or light purple-pink to deep red, masked with irregular pattern of dark pigmentation that varies between specimens from barely detectable grey through brown to melanistic black. Masking nearly absent on one large individual with red coloration. Some individuals had faded, nearly colorless appearance. Pattern of black pigmentation on sides below dorsal fin basically three large irregular areas extending from midline to or onto fin. One pigmented area beneath middle and another beneath last 1/4 of spinous dorsal fin—first slightly interrupted by narrow light area where it crosses lateral line; second broadly interrupted by light band reminiscent of band along lateral line of *S. proriger*. Third large pigmented area beneath soft dorsal fin forms irregular circle with light spot at center, continues onto lower 1/2 of soft dorsal fin, interrupted near lower border by broad light-colored band along lateral line. Band extends over posterior 2/3 of body. Small pigmented area on dorsal caudal peduncle extends downward to but not across lateral line and forms anterior boundary of pupil-sized lighter spot on dorsal shoulder of caudal fin. Head with diffuse brown to black shading from symphyseal knob and nearby portions of lower jaw to nape; on snout, shaded area extends ventrally to a line through 7 o'clock position on eye. Behind eye, dark shading extends ventrally to about 5 o'clock position and posteriorly over preopercle. Nape dusky to a line between 1 o'clock position of eye and anterior lateral line. Opercle with two broad pigmented bands whose converging axes meet when projected into lower hemisphere of eye. Upper band includes both opercular spines; lower extends toward pectoral base and includes upper two preopercular spines. Lower band aligns generally with irregular pigmented areas on upper 1/2 of pectoral base. On cheek of most specimens a short pigmented streak extends posteriorly and slightly downward from upper

corner of maxilla. Lower cheeks, jaws, and opercular membranes suffused with pink, rose-pink, or red. Spinous dorsal fin with distal 1/2 to 2/3 of interspinous membranes black; remainder above back has pink-related background color of body. Dark pigmentation absent in skin over fin spines, contrasting with dark interspinous membranes. Soft dorsal fin usually distinguished by continuation of body pigmentation onto its lower half where pigmentation forms flattened upper 1/2 of irregular doughnut- or tire-shaped mark. Distally on soft dorsal fin, interspinous membranes darkly pigmented near rays; intervening areas some shade of translucent pink or red. Dark pigmentation terminates equally along fin to form translucent pink border. Caudal fin membranes almost entirely black. Caudal fin terminates with narrow clear or translucent pink or red border. Anal fin pink or red, usually with narrow area or streak of black pigmentation between spines II and III; soft-rayed portion has dusky pigmentation on distal 1/2 of membranes. Background color of pectoral fin same as body but masked by dusky pigmentation in large circular or oval area on upper 2/3 of fin. Small dark pupil-sized spot usually near insertion of rays near midline of fin. Pelvic fin colored as belly.

In preserved specimens dark areas of head, body, and fins persist and include black membranes in spinous dorsal and caudal fins, black stripe between anal spines II and III, dusky pigmentation of lower jaw tip, radiating bands on opercle, black spot in center of pectoral fin, doughnut- or tire-shaped mark below soft dorsal fin, and broad light stripe that accompanies lateral line over posterior 2/3 of body and interrupts two of three large dark areas on body sides. Peritoneum normally dark brown to jet black (one specimen had brown peritoneum with black spots); a melanistic specimen (Figure 3) had light brown peritoneum with black spots).

MERISTIC CHARACTERS

See Table 1.

SIZE OF BODY PARTS

Metrical data on 30 characters are summarized in Tables 2-4. Two methods for fitting regressions of measurements on standard length

TABLE 1.—Counts for meristic characters for holotype of *Sebastes variegatus* and ranges of frequent and infrequent counts for all specimens.

Character	Holotype	Specimens including holotype	
		N	Counts and their frequencies (*N*)
Precaudal vertebrae	10	3	10
Caudal vertebrae (including urostyle)	17	3	17
Dorsal fin spinous rays	13	38	13(36); 14(2)
Dorsal fin soft rays	14	38	13(1); 14-15(37)
Total rays in dorsal fin	27	38	27-28
Anal fin soft rays	7	38	[1]7
Lower single pectoral rays (2 sides)	8, 9	75	7(3); 8-9(72)
Total pectoral rays (2 sides)	18, 18	75	17(4); 18(70); 19(1)
Pored lateral line scales on body (2 sides)	49, 51	73	42(1); 43-51(71); 52(1)
Pored lateral line scales on caudal fin (2 sides)	1, 1	73	0(1); 1(70); 2(2)
Scale rows below lateral line (2 sides)	58, 58	45	46(1); 47-57(42); 58(2)
Rakers on upper limb	11	38	9(1); 10-12(37)
Rakers on lower limb, including raker at bend	28	38	26-28(37); 29(1)
Total rakers	39	38	36-40(37); 41(1)

[1] One of two additional specimens, examined subsequently, had 6 soft rays in the anal fin.

TABLE 2.—Measurements (mm) of type material, *Sebastes variegatus*.

Character	Holotype (SL = 201)	Paratypes	
		No. 1 (SL = 185)	No. 2 (SL = 193)
Head length	70.0	63.0	64.6
Upper jaw length	32.3	28.8	29.3
Prenarial pore length	1.7	1.0	1.4
Subnarial pore length	2.4	2.5	2.5
Orbit diameter	22.4	18.5	19.0
Interorbital width	14.2	13.4	13.7
Raker length	12.0	10.7	12.0
Lower jaw projection	3.8	3.3	3.6
Snout length	17.5	16.4	17.0
Suborbital width	2.5	2.7	2.2
Head width	30.2	29.5	29.8
Pectoral fin length	60.0	53.1	56.7
Pectoral base length	19.0	18.3	19.2
Caudal peduncle depth	18.0	15.9	16.0
Dorsal caudal peduncle length	28.7	26.0	26.5
Ventral caudal peduncle length	44.3	40.2	40.9
Body depth (pelvic)	58.4	56.5	56.5
Body depth (anal)	47.5	45.2	48.9
Anal spine I length	18.5	16.9	16.5
Anal spine II length	40.2	36.6	37.8
Anal spine III length	33.4	30.9	30.8
Soft dorsal fin base length	52.3	43.7	45.3
Anal fin base length	32.1	27.6	31.0
Pelvic fin spine length	31.7	28.8	30.3
Pelvic fin length	48.0	42.7	44.5
Pelvic insertion to midvent	45.4	46.5	45.6
Midvent to anal fin	11.5	11.0	11.3
Longest dorsal fin spine length	29.8	29.3	28.2
Longest dorsal soft ray length	29.2	26.7	27.2
Longest anal soft ray length	37.9	35.7	35.4

TABLE 3.—Regression of measurements (Y) on SL (X) in the point-slope form of the log-transformed allometric equation $Y = aX^b$ over the interval $109 < \text{SLmm} < 286$, *Sebastes variegatus*.

Character (No. specimens)	$\bar{X}$	$\bar{Y}$	$b(\pm 95\%)$[1]	S_{yx}	r^2
Head length (38)	2.26567	1.80164	1.03704(±0.02555)*	0.0100	0.995
Upper jaw (38)	2.26567	1.46445	1.00526(±0.03700)n.s.	0.0100	0.988
Prenarial pore (38)	2.26567	0.12076	0.68961(±0.22125)*	0.4499	0.526
Subnarial pore (38)	2.26567	0.36358	0.64076(±0.15785)*	0.0510	0.653
Orbit (38)	2.26567	1.29809	0.89650(±0.07000)*	0.0224	0.949
Interorbital (38)	2.26567	1.10458	1.17465(±0.05915)*	0.0200	0.978
Raker (37)	2.26051	1.02983	0.99778(±0.09776)n.s.	0.0300	0.926
Lower jaw projection (35)	2.26552	0.59671	1.40000(±0.18610)*	0.0583	0.876
Snout (35)	2.26552	1.20640	1.05453(±0.05560)n.s.	0.0173	0.978
Suborbital (35)	2.26552	0.39634	0.76861(±0.17185)*	0.0539	0.714
Head width (34)	2.27223	1.47624	1.03266(±0.07375)n.s.	0.0224	0.962
Pectoral fin (35)	2.26539	1.71905	0.96682(±0.04611)n.s.	0.0115	0.982
Pectoral base (38)	2.26539	1.24749	0.95907(±0.04845)n.s.	0.0144	0.979
Caudal peduncle depth (35)	2.25292	1.21462	0.93501(±0.05720)*	0.0173	0.971
Dorsal caudal peduncle (35)	2.26552	1.42222	0.98447(±0.06855)n.s.	0.0224	0.962
Ventral caudal peduncle (35)	2.26552	1.59340	0.92085(±0.04905)*	0.0141	0.978
Body depth (pelvic) (38)	2.26567	1.74993	1.01305(±0.06455)n.s.	0.0200	0.966
Body depth (anal) (35)	2.26552	1.66765	0.89023(±0.06100)*	0.0200	0.964
Anal spine I (38)	2.26567	1.20583	0.81995(±0.10465)*	0.0332	0.876
Anal spine II (37)	2.26051	1.54643	0.73519(±0.06340)*	0.0200	0.940
Anal spine III (35)	2.26552	1.46436	0.83416(±0.05255)*	0.0173	0.968
Soft dorsal base (35)	2.26552	1.63887	0.90102(±0.10955)n.s.	0.0346	0.894
Anal base (35)	2.26552	1.46978	0.81406(±0.07225)*	0.0224	0.941
Pelvic fin spine (34)	2.26648	1.45182	0.85218(±0.04725)*	0.0141	0.976
Pelvic fin (38)	2.26567	1.62047	0.93185(±0.03595)*	0.0100	0.987
Pelvic insertion to midvent (38)	2.26567	1.63481	1.15544(±0.11405)*	0.0361	0.922
Midvent to anal fin (35)	2.26552	1.00595	1.10201(±0.25370)n.s.	0.0794	0.703
Longest dorsal fin spine (36)	2.26180	1.40658	0.94004(±0.09285)n.s.	0.0283	0.926
Longest dorsal soft ray (34)	2.26648	1.42402	0.96599(±0.08310)n.s.	0.0265	0.945
Longest anal soft ray (35)	2.26552	1.54610	0.97441(±0.04500)n.s.	0.0141	0.983

[1] Asterisk indicates a difference in the slope exponent (b) significantly different from unity at the 95% level; n.s. indicates no significant difference at this confidence level.

were explored, the power or allometric equation, $Y = aX^b$, in $\log_{10}$ transformed form, and the first degree or rectilinear equation, $Y = a + bX$, untransformed. Both functions were fit by a computer regression program (Sokal and Rohlf, 1969:696). Degree of fit was judged by the "coefficient of percentage variation explained by regression," the square of the correlation coefficient for regression, r^2 (Table 3). In 18 of the 30 characters, nearly identical r^2's were obtained by the alternative equations, including 9 characters for which the allometric exponent differed in a minor (<0.20) but significant degree from unity at the 95% confidence level, i.e. the growth relationships seemed to depart significantly from isometry. Plots of selected examples from the nine characters showed only minor differences between the two functions in these instances. In four comparisons, three of which showed significant departure from unity in the allometric exponent (subnarial pore, lower jaw projection, and suborbital), the first-degree equation appeared to give a superior fit. For the remaining eight comparisons, the allometric equation was superior.

In the light of these considerations, the $\log_{10}$ transformations of the allometric equation ($\log Y = \log a + b \log X$) was chosen for general application because it is more versatile and gave a satisfactory fit in all comparisons. Those few characters which were fit somewhat better by the first-degree equation appear to be of relatively minor utility in rockfish systematics. Parameters are presented in the point-slope form of the transformed allometric equation ($Y = \bar{Y} + b\ (X - \bar{X})$, where $Y = \log_{10} Y$ and $X = \log_{10} X$) to emphasize the working interval about the means of the variates and to de-emphasize the Y-intercept which has no theoretical value in these representations.

The measurements are also presented in the form of 95% confidence limits for proportions of future individual specimens, based on the material examined (Table 4). The limits were

TABLE 4.—Upper and lower 95% confidence limits for single future observations on proportions (percent of SL), based on the material examined.[1]

Character	Standard length (mm)				
	120	160	200	240	280
Head length	35.52	35.84	36.13	36.40	36.64
	32.19	32.59	32.86	33.06	33.22
Upper jaw	16.56	16.56	16.58	16.61	16.64
	15.01	15.06	15.08	15.08	15.08
Prenarial pore	1.16	1.05	0.98	0.93	0.89
	0.58	0.53	0.50	0.47	0.44
Subnarial pore	1.88	1.68	1.55	1.46	1.39
	1.14	1.04	0.96	0.89	0.84
Orbit	12.57	12.16	11.88	11.67	11.52
	10.09	9.83	9.61	9.42	9.25
Interorbital	7.06	7.40	7.70	7.96	8.19
	5.80	6.12	6.37	6.57	6.73
Raker	6.82	6.78	6.78	6.79	6.81
	5.08	5.10	5.10	5.08	5.07
Lower jaw projection	2.41	2.68	2.92	3.16	3.38
	1.35	1.53	1.68	1.80	1.90
Snout	9.29	9.41	9.52	9.63	9.73
	7.83	7.97	8.07	8.14	8.20
Suborbital	1.95	1.81	1.71	1.65	1.60
	1.14	1.08	1.03	0.98	0.94
Head width	17.63	17.71	17.83	17.97	18.11
	14.10	14.30	14.41	14.48	14.51
Pectoral fin length	30.52	30.17	29.95	29.79	29.67
	27.23	27.03	26.83	26.65	26.48
Pectoral base	10.46	10.32	10.22	10.15	10.11
	9.11	9.02	8.94	8.86	8.80
Caudal peduncle depth	10.24	10.02	9.87	9.77	9.70
	8.63	8.49	8.37	8.26	8.16
Dorsal caudal peduncle	16.13	16.00	15.94	15.91	15.91
	12.93	12.92	12.88	12.83	12.76
Ventral caudal peduncle	23.60	23.02	22.61	22.31	22.07
	20.52	20.11	19.76	19.46	19.20
Body depth (pelvic)	33.46	33.48	33.56	33.69	33.83
	27.49	27.68	27.77	27.80	27.80
Body depth (anal)	29.21	28.20	27.51	27.01	26.61
	23.97	23.30	22.75	22.27	21.84
Anal spine I	11.08	10.46	10.05	9.74	9.51
	8.00	7.64	7.34	7.09	6.87
Anal spine II	23.81	21.99	20.72	19.77	19.03
	19.55	18.18	17.14	16.31	15.62
Anal spine III	18.49	17.58	16.93	16.45	16.06
	15.58	14.90	14.36	13.92	13.54
Soft dorsal fin base	29.25	28.26	27.63	27.20	26.89
	20.77	20.31	19.87	19.47	19.11
Anal fin base	21.70	20.39	19.43	18.68	18.07
	13.85	13.24	12.79	12.43	12.14
Pelvic fin spine	17.52	16.75	16.20	15.78	15.45
	15.23	14.63	14.16	13.77	13.44
Pelvic fin length	24.68	24.17	23.80	23.52	23.30
	22.38	21.98	21.65	21.37	21.12
Pelvic insertion to midvent	26.13	27.16	28.11	28.98	29.80
	18.33	19.28	19.97	20.50	20.92
Midvent to anal fin	7.80	7.92	8.09	8.29	8.49
	3.56	3.71	3.80	3.85	3.88
Longest dorsal fin spine	16.46	16.10	15.88	15.74	15.64
	12.45	12.30	12.14	11.98	11.83
Longest dorsal soft ray	16.63	16.39	16.26	16.19	16.15
	12.79	12.73	12.64	12.54	12.44
Longest anal soft ray	20.69	20.49	20.37	20.29	20.24
	17.99	17.90	17.80	17.70	17.61

[1] Based on confidence belts for individual predicted measurements (Sokal and Rohlf, 1969:422) from the parameters for regression (Table 3). Data were back-transformed to arithmetic values for computation of proportions. If limits are desired in the form of measurements, the percentages may be multiplied by the appropriate SL.

obtained from 95% confidence limits for fits to regression (Table 3) back-transformed to the original variates and converted to percent of standard length. Marr (1955) discussed the disadvantages of using proportions in systematics, particularly when size-specific changes are not identified, but neglected several advantages. Size-specific changes are here identified, and the presentation of data in the form of proportions facilitates rapid comparisons between specimens and allows data on measurements to be presented more economically than in graphs. Proportions vary much less with length than original measurements, sometimes surprisingly little when the numerous factors that influence their variation are considered (Table 4), and therefore allow easier interpolation between tabulated reference points than representations of original variates. Also, the continued use of proportions in fish systematics, often without indication of size-specific changes, attests to a prevailing opinion that proportions are useful despite their occasional misuse and other drawbacks.

Plots of the limits for proportions (Table 4) disclose that they are slightly curvilinear and asymmetrical from left to right. These characteristics reflect the y-intercept effect discussed by Marr (1955) combined with the effects of normal divergence of confidence belts in regression with distance from the combined mean, allometry between the original variates, and distortion caused by back-transformation from the logarithm of a function. Size-specific confidence limits for proportions whose allometric coefficients did not differ significantly from unity (Table 3) are included in Table 4 because the calculated value is a better estimate of the exponent than is arbitrarily assumed isometry.

AXIAL SKELETON

X-rays of types show 27 vertebrae, including urostyle (Table 1). Pterygiophores of spinous dorsal fin single in spaces between neural spines except between neural spines 2 and 3, which contains pterygiophores of dorsal fin spines 2 and 3. Pterygiophores of soft dorsal fin usually doubled in interneural spaces except single pre-

ceding neural spines 5 and 8 or 9. Caudal skeleton apparently with hypurals 2 and 3 and 4 and 5 fused into upper and lower plates, respectively, as determined for other scorpaenid representatives by Quast (1965:580). Point of enlarged pterygiophore that supports anal fin spines I and II contacts haemal arch of 11th vertebra, here considered the first caudal vertebra.

ELECTROPHORETIC PATTERN

A standardized starch gel electrophaerogram of haemoglobin from a male adult *S. variegatus* (BC 70-2, from Queen Charlotte Sound, British Columbia), with comparative material on *S. zacentrus*, was kindly furnished by Henry Tsuyuki of the Fisheries Research Board of Canada (Figure 4).[3]

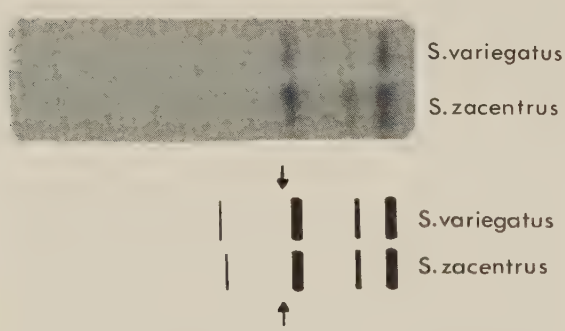

FIGURE 4.—Comparative starch gel electrophaerograms of the haemoglobins from *S. variegatus* and *S. zacentrus* furnished by Henry Tsuyuki, Fisheries Research Board of Canada (see text). The arrow represents the origin in the schematized pattern. The anode is to the right and the cathode to the left. The minor cathodal zone, which Tsuyuki found to be consistently diagnostic for the two species, could not be reproduced photographically and is shown in schematic form in approximately the concentrations found.

[3] Vancouver Laboratory, 6640 NW. Marine Drive, Vancouver 8, British Columbia, 28 July 1970. "Erythrocytes were washed once with a 1% sodium chloride solution before hemolysis to avoid contamination from serum proteins. Haemoglobins from washed and unwashed red cells possessed identical patterns." For methods used in obtaining blood samples and for electrophoresis, see Tsuyuki et al. (1968). Identity of the *S. variegatus* specimen verified by the author; however, the specimen had a head size much larger than normal (38.3% of SL) and was the only specimen out of 40 seen that had 6 soft rays in the anal fin instead of 7.

SIMILAR SPECIES

Sebastes variegatus resembles *S. emphaeus, S. proriger, S. saxicola, S. wilsoni,* and *S. zacentrus* in morphology, morphometry, meristics, and coloration. The six species appear to be closely related members of a complex that generally shares the characters of *S. variegatus* other than the pectoral count of 18, the unpigmented band that encloses the lateral line and extends about 2/3 along the body sides, and the black coloration of the spinous dorsal and caudal fin membranes. The complex may also include *S. dalli, S. elongatus, S. jordani,* and *S. semicinctus* which also seem to resemble the six species, but these appear to be easier to differentiate from *S. variegatus* and I do not further consider them here. Although the analyses of rockfish haemoglobin and muscle proteins by Tsuyuki et al. (1968) shed little light on the group's validity, subsequent work by Tsuyuki (see footnote 3) suggests a close relationship between *S. variegatus* and *S. zacentrus* (Figure 4). The possibility that *S. variegatus* represents a hybrid may be dismissed because of a normal degree of variation in measurements, distinctiveness and consistency of pectoral ray counts and body coloration, abundance of specimens, and normal reproduction as indicated by normally developed gonads and gravid females.

As a natural group, the six species do not appear to coincide with those at the subgeneric level erected by Cramer, Eigenmann, and Beeson, or Jordan and Evermann, as described by Jordan and Evermann (1898, II: 1765-1777). However, in some respects it does resemble the subgenus *Hatumeus*, erected by Matsubara (1943b: 192) under *Sebastes*, and the type species, *Sebastodes owstoni*, as figured by Jordan and Thompson (1914:pl. 31, fig. 3).

Ranges of meristic characters for most species in the hypothetical group (from Phillips (1957) plus personal examination of representatives—Table 5) either are nearly identical (soft rays in the dorsal, anal, and pectoral fins) or overlap broadly (pored scales in the lateral line, gill rakers, and scale rows below lateral line). In characteristics for which most of the group are nearly identical, *S. variegatus* deviates mark-

TABLE 5.—Data and material for species comparisons with *Sebastes variegatus*.

Species	Phillips (1957)			Material examined		
	No. specimens	Meristics		No. specimens	Meristics	
		No.	SL		No.	SL
S. emphaeus	--	--	--	7	7	101-131
S. proriger	7	6	230-389	12	12	161-270
S. saxicola	35	9	103-308	--	--	--
S. wilsoni	3	3	102-133	6	6	50-174
S. zacentrus	15	10	166-338	20	20	133-281

edly only in pectoral count. In those species where means of characteristics differ noticeably but ranges overlap broadly, *S. variegatus* usually is of central value, which suggests that it may be a more generalized member of the group.

Comparisons of morphometric characters between *S. variegatus* and the other species lead to a similar conclusion. Ranges for the species overlap broadly, based on Phillips (1957) and my own measurements (head length, longest dorsal fin spine, least depth of caudal peduncle, pelvic insertion to vent, raker at bend of gill arch, width of pectoral fin base, body depth at pelvic girdle, bony interorbital, orbit, length of pectoral fin, length of pelvic fin, and length of upper jaw—in percent of standard length).

To assist differentiating *S. variegatus* from similar species a table of discriminatory characters is presented (Table 6).

GEOGRAPHIC AND BATHYMETRIC RANGE

Present known range of *S. variegatus* is from Unimak Pass, Aleutian Islands, to Goose Island Bank, Queen Charlotte Sound[4] (Figure 5). Depth of capture ranges from 70 to 305 m.

ACKNOWLEDGMENTS

William I. Follett (California Academy of Sciences), Warren C. Freihofer (Stanford University), and Norman J. Wilimovsky (University of British Columbia) gave permission for examination of collections or shipped material.

[4] The record for Queen Charlotte Sound was obtained by Sigurd J. Westrheim, Fisheries Research Board of Canada, Nanaimo Research Station, Nanaimo, British Columbia, on the RV *G. B. Reed* during Cruise GBR 70-1 (personal communication, 5 August 1970).

TABLE 6.—Summary of salient comparative features of *Sebastes variegatus* with five similar speces. (Proportions of *S. variegatus* refer to 95% confidence limits of Table 4. Source and quantity of data on other species are summarized in Table 5. Code for sources: *, Phillips (1957); **, original material (Tables 1-4); ***, both sources. In some instances my data on northeastern Pacific specimens differ from Phillips (1957) in degree of discrimination.)

S. emphaeus.
Counts: Pectoral rays 17 (occas. 18)**, *variegatus* 18 (occas. 17); scale rows ≤46*, *variegatus* ≥46. Percent of SL (apply to interval of size overlap, 109-131 mm): Upper jaw ≤15.0**, *variegatus* ≥15.0; ventral caudal peduncle ≤20.8**, *variegatus* ≥20.5; anal spine I ≤8.3**, *variegatus* ≥8.0; anal spine II ≤19.4**, *variegatus* ≥19.6; anal spine III ≤15.8**, *variegatus* ≥15.6. Pigmentation: Blotches on sides not interrupted by an unpigmented band along lateral line; no black area on fin membrane between anal spines II and III (both characteristics from pl. 31 of Starks, 1911).

S. proriger.
Counts: Pectoral rays 17*** (occas. 18)**, *variegatus* 18 (occas. 17); scale rows ≥55* (47-56)**, *variegatus* ≤58. Percent of SL (apply to interval of size overlap 161-270 mm, for Quast data): Upper jaw ≤14.7* (≤15.7)**, *variegatus* ≥15.1; orbit ≤9.3* (≤10.5)**, *variegatus* ≥9.3; anal spine II ≤15.4* (≤16.4 but normally below 95% limits of *variegatus* at size [Table 4])**; anal spine III (below 95% limits of *variegatus* at size [Table 4])**; pelvic fin (below 95% limits of *variegatus* at size [Table 4])**, longest dorsal soft ray ≤12.5*, *variegatus* ≥12.4; longest anal soft ray ≤16.1*, *variegatus* ≥17.6. Pigmentation: Unpigmented band along lateral line extends to head.

S. saxicola.
Counts: Dorsal soft rays 12 (occas. 13)*, *variegatus* 14 or 15 (occas. 13); pectoral rays 16 (occas. 15 or 17)*, *variegatus* 18 (occas. 17); lateral line pored scales ≤42*, *variegatus* ≥42; rakers ≤34*, *variegatus* ≥36. Percent of SL: head ≥35.7*, *variegatus* ≤36.6; orbit ≥11.8*, *variegatus* ≤12.5; longest anal soft ray ≤18.2*, *variegatus* ≥17.6. Pigmentation: Blotches on sides not interrupted by unpigmented band along lateral line.

S. wilsoni.
Counts: Anal soft rays 6***, *variegatus* 7 (rarely 6); pectoral rays 16-17* (occas. 18)**, *variegatus* 18 (occas. 17); lateral line pored scales ≤41* (≤43)**, *variegatus* ≥42; scale rows 45-50* (41-45)**, *variegatus* ≥46. Percent of SL (apply to interval of size overlap, 109-174 mm, in Quast data): pelvic fin length ≤22.0**, *variegatus* ≥22.0. Pigmentation: Dark blotches on sides not interrupted by an unpigmented band along lateral line.

S. zacentrus.
Counts: Pectoral rays 17 (occas. 18)*, (occas. 19)**, *variegatus* 18 (rarely 17 or 19)**; scale rows 43-50* (≤47)**, *variegatus* ≥46; rakers ≤37* (≤38)**, *variegatus* ≥36. Percent of SL (apply to interval of size overlap, 133-281, for Quast data): Upper jaw 15.6-16.9* (normally above 95% limits of *variegatus* at size [Table 4])**; longest anal soft ray ≤17.9*, *variegatus* ≥17.6. Pigmentation: Blotchts on sides not interrupted by unpigmented band along lateral line.

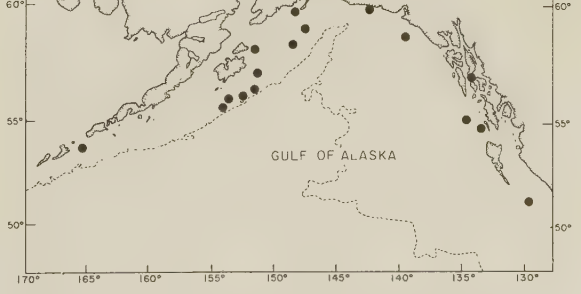

FIGURE 5.—Localities at which *Sebastes variegatus* were captured.

Sigurd J. Westrheim (Fisheries Research Board of Canada, Nanaimo Research Station) furnished fresh material and reviewed the manuscript, and Henry Tsuyuki (Fisheries Research Board of Canada, Vancouver Laboratory) contributed the haemoglobin electrophaerograms. Daniel M. Cohen (United States National Museum), William N. Eschmeyer (California Academy of Sciences), and Bruce L. Wing (National Marine Fisheries Service Biological Laboratory, Auke Bay, Alaska) provided editorial and scientific comments.

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CALICO SCALLOP DISTRIBUTION, ABUNDANCE, AND YIELD OFF EASTERN FLORIDA, 1967-68[1]

RICHARD B. ROE,[2] ROBERT CUMMINS, JR.,[3] AND HARVEY R. BULLIS, JR.[4]

ABSTRACT

During 18 months, from August 1967 to December 1968, the National Marine Fisheries Service Exploratory Fishing and Gear Research Base in Pascagoula, Miss., conducted a comprehensive survey of the calico scallop (*Argopecten gibbus*) grounds off eastern Florida. The survey disclosed various aspects of the life history, distribution, abundance, and yield and annual variation in geographical and depth distribution. Predictions for a fall fishery are possible since distribution and abundance are established at spat set and can be delineated by midsummer. A fall fishery is recommended as catch rates and yield were highest between September and December and decreased rapidly during late winter and spring.

There are two or more species of *Argopecten* called "calico" scallops (Waller, 1969); however, the more common is *A. gibbus* (L.). This is the species involved in our study, occurring from Delaware Bay to the Caribbean Sea and throughout the Gulf of Mexico (Johnson, 1934; Bullis and Ingle, 1959; Carpenter, 1967; Waller, 1969). Calico scallops derive their name from the blotched coloration of their shells which vary from red to light brown on a white background, giving a "calico" effect to the shell.

Three commercial grounds have been delineated by the Exploratory Fishing and Gear Research Base at Pascagoula, Miss., and its Field Station in Brunswick, Ga. These are located off North Carolina (Cummins, Rivers, and Struhsaker, 1962), off eastern Florida near Cape Kennedy (Bullis and Cummins, 1961; Cummins, et al., 1969;[5] Drummond, 1969), and in the northeastern Gulf of Mexico (Bullis and Ingle, 1959; Carpenter, 1967). Explorations had shown a large resource exists, particularly off Florida, but monthly and yearly changes in distribution and abundance had seriously hampered delineation of the resource.

In August 1967 an 18-month survey was initiated on the Cape Kennedy grounds to assess the scallop stock and determine the causes of variation in distribution, abundance, and yield. These grounds were selected because of size, location, and a developing industry.

The survey provided information on the dynamics of the scallop population present at that time, but there is a need for additional studies on life history, age, and growth.

METHODS

Cruises were conducted monthly between August and December 1967, and bimonthly between February and December 1968. Four standard transects, each extending from 10 to 40 fm, were made on each cruise. These were: transect A at lat 28°03′ N, transect B at lat 28°27′ N, transect C at lat 29°03′ N, and transect D at lat 29°25′ N (Figure 1). Tows were also made between transects. An 8-ft scallop dredge, fitted with 2-inch bag rings and 2½-inch mesh nylon liners, was used in the survey. All tows were 30 min.

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[4] National Marine Fisheries Service, Washington, D.C. 20240.

[5] Cummins, R., Jr., R. Maurer, L. May, and J. Rivers. 1969. Summary log of scallop locations with predicted catch rates of Cape Kennedy grounds — fall 1969. Issued by Bureau of Commercial Fisheries Exploratory Fishing and Gear Research Field Station, Brunswick, Ga., for limited distribution.

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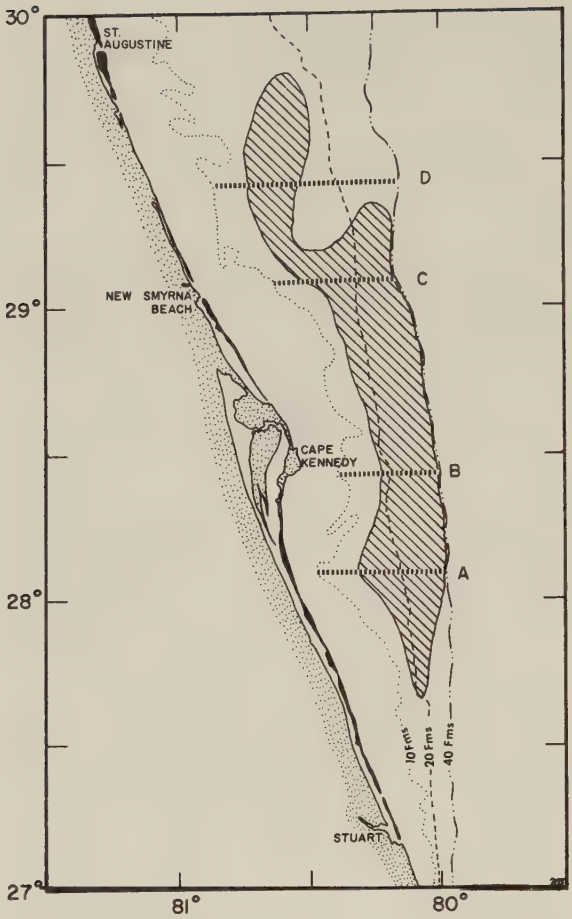

FIGURE 1.—General distribution of calico scallops and four standard transects occupied during the 1967-68 survey off the Florida east coast.

A total of 1,483 drags was made during the survey—285 in transect A, 403 in transect B, 493 in transect C, and 302 in transect D. The following were determined for each drag: total catch in bushels (using a standard steel shrimp basket averaging 70 lb.), number of bushels of live scallops, pounds of meat per bushel, number of pints of meat per bushel, number of meats per pint, and size frequency (measured as shell diameter in millimeters). Randomly selected individuals were examined for gonad coloration and sexual maturation. The data were analyzed with the UNIVAC 9200 computer at the Exploratory Fishing and Gear Research Base at Pascagoula, Miss.

The survey results are subdivided according to spawning, age and growth, mortality, distribution and abundance, and yield.

SPAWNING

Bourne and Bligh (1965) found sea scallop (*Placopecten magellanicus*) ovaries change color during maturation, progressing from pink at early stages to deep coral-red when ripe. Color changes were also noted in calico scallop ovaries, undeveloped ovaries being whitish, ripe ovaries bright reddish-orange.

Yellowish-orange coloration was seen in very few scallops during the August cruise, but by September the rate and incidence of color change was highly noticeable. Coloration and incidence increased into fall and winter and by February ovaries were predominantly reddish-orange or ripe. The majority of ovaries taken in April were bright reddish-orange. June ovaries were largely uncolored.

Sexual maturation in sea scallops begins in March, spawning occurring 7 months or so later in September or October. Calico scallops apparently have a similar maturation period based on color change progression which begins in August and ends in March or April. Protracted spawning does take place in some areas since small numbers of 5 to 30 mm scallops occur throughout the year (Figure 2).

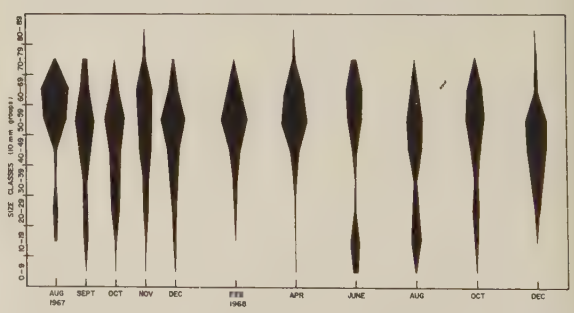

FIGURE 2.—Monthly size/frequency distribution of scallops for the combined transects off the Florida east coast during the 1967-68 survey.

AGE AND GROWTH

Monthly size frequencies are shown in Figure 2. From June until September the distribution is bimodal, but in October the lower mode disappears and a single mode exists until April. This is clearly illustrated in the 1968 data beginning in June when modes occur at 11.9 and 62.1 mm. In August modes are present at 26.0 and 60.6 mm and in October at 34.0 and 61.5 mm. Only one mode at 51.0 mm occurs in December.

The lower mode value gradually increases during this period while the upper mode decreases. These changes reflect a melding of year classes caused by a more rapid growth rate in the younger year class than in the older one. In December the two year classes are indistinguishable. Since year classes cannot be separated in the winter data, it is difficult to determine when the older class disappears. Presumably this occurs some time between December and April.

Growth rates could not be accurately determined from the data because of gear selectivity for the smaller sizes and because year classes were often inseparable in the size-frequency distribution. A growth estimate was derived for a selected bed located on transect C in 22 to 27 fm. The size-frequency data indicate two year classes were present most of the survey period (Table 1). These are more distinct in the separated 1968 data (Table 2).

The mean size increases linearly by about 1.0 to 2.0 mm per month during August to February (Table 1). From April to December the mean gradually decreases because of recruitment from the spring spawn.

TABLE 2.—Monthly size frequency data for transect C, 22 to 27 fm, divided by year class. Classes are 10 mm.

Size class	Year class A				Year class B			
	June	Aug.	Oct.	Dec.	June	Aug.	Oct.	Dec.
0-9	6	1	--	--	--	--	--	--
10-19	24	57	2	--	--	--	--	--
20-29	4	163	61	125	--	--	--	--
30-39	--	41	3	260	--	--	--	--
40-49	--	--	--	116	1	--	--	--
50-59	--	--	--	103	44	41	8	--
60-69	--	--	--	--	72	211	103	138
70-79	--	--	--	--	31	38	21	7
Mean	13.9	23.8	25.7	37.8	63.5	64.4	65.5	65.0

Since the age groups interact the means given in Table 1 actually represent a combined year class average. Therefore, the June to December 1968 data have been separated into two year classes based on size (Table 2). Year class A was spawned in the spring of 1968 and year class B, representing the larger sizes, was spawned in the spring of 1967. The monthly mean for year class A increased curvilinearly 24 mm from June to December. Year class B mean increased only 2.0 mm from June to October then decreased slightly in December. The increase appeared linear.

Both year classes show evidence of a sigmoid growth curve. Gibson (1956) gives a sigmoid curve for *Pecten maximus* where time is measured in years rather than months as in calico scallops.

MORTALITY

Calico scallops on the Cape Kennedy grounds experienced light commercial exploitation prior

TABLE 1.—Monthly size frequencies for transect C, 22 to 27 fm, by 10-mm size classes, 1967-68.

Size class	1967					1968					
	Aug.	Sept.	Oct.	Nov.	Dec.	Feb.	Apr.	June	Aug.	Oct.	Dec.
0-9	--	--	--	--	--	--	--	6	1	--	--
10-19	1	4	--	--	--	--	--	24	57	2	--
20-29	1	4	1	1	--	--	--	4	163	61	125
30-39	1	3	--	3	--	3	--	--	41	3	260
40-49	2	16	3	7	--	4	38	1	--	--	116
50-59	11	58	72	23	38	295	185	44	41	8	103
60-69	9	42	43	39	25	567	343	72	211	103	138
70-79	2	18	2	7	1	28	46	31	38	21	7
80-89	--	--	--	--	--	--	1	--	--	--	--
Mean	55.2	56.4	57.9	59.1	58.7	61.3	61.0	54.2	45.1	51.9	43.0

to 1967. Landings in 1967 totaled approximately 5,000 bu and although several vessels fished the area in 1968, the overall catch was light. Mortality during the survey period was therefore assumed largely due to natural causes.

Catch curves using Ricker's (1958) method of plotting $\log_e$ (average number per drag per month) were constructed (Figure 3). A linear

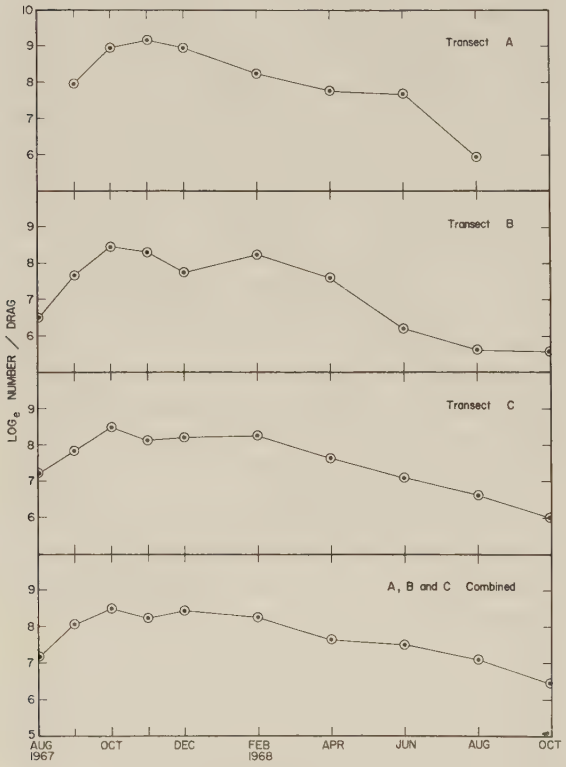

FIGURE 3.—Catch curves for transects A, B, C, and combined.

regression was applied to the descending curve according to Beverton and Holt (1957), using b as an estimate of $F + X$. Since F was negligible, b is an estimate of X or natural mortality. The instantaneous mortality rate (i) is equal to b and the monthly mortality rate (a) was derived from Ricker's appendix.

Data used in the analysis represented the 1967 year class except in transect D where catch data were too sparse for analysis. December 1968 data were not available except for transect C. October 1968 was omitted from transect A because of insufficient data.

The ascending left limb of the curve reflects recruitment. Mortality during this period is difficult to determine because small scallops are inaccessible to the dredge. The dome varied among transects but occurred from October to December or February. The descending limb extended from December to October (August in transect A). All limbs are curvilinear but vary in shape. The curve for transect A is variable. That for transect B is concave from February to June then becomes convex. Transect C curve is almost linear.

Monthly mortality rates, computed assuming a linear relationship (as in Beverton and Holt, 1957), are given in Table 3. Two time periods

TABLE 3.—Monthly mortality rates (a) and instantaneous mortality rates (i) for transects A, B, and C, individually and combined, for various time periods.

Transect	Time period	i	a
			%
A	10/67-12/67	−0.012	1
	12/67- 8/68	−0.327	27
B	10/67-12/67	−0.365	31
	12/67-10/68	−0.286	25
C	10/67-12/67	−0.150	14
	12/67-10/68	−0.236	21
COMBINED	10/67-12/67	−0.020	2
	12/67-10/68	−0.189	18
C (22-27 fm)	2/68-10/68	−0.231	21

were treated: one from October to December (the optimum fishing season) and the other from December to October (the descending limb). The latter period includes the spawning season. Although accurate rates for the first period (the dome) could not be accurately ascertained, a crude mortality estimate was desired for the fishing season.

Monthly mortality rates ranged from 1% to 31% for the dome and 18% to 27% for the descending limb. Averages were 12% and 23% respectively.

Age classes were difficult to distinguish and all data may not have been from the 1967 year class. This could have caused differences in the

catch curves. To test this possibility, data from a bed of known age composition were used to construct a catch curve. This bed, located at lat 29°16′ N in 22 to 27 fm on transect C, was established in August 1967 and can be accurately traced through October 1968 with length-frequency data.

The catch curve for the bed (August 1967 to October 1968) is given in Figure 4. Recruitment in late summer and fall was strong. Recruitment and mortality seem balanced from October to February, but neither can be accurately determined. The convex descending limb (February to October) indicates a nonuniform mortality rate, increasing rapidly after June with the termination of spawning.

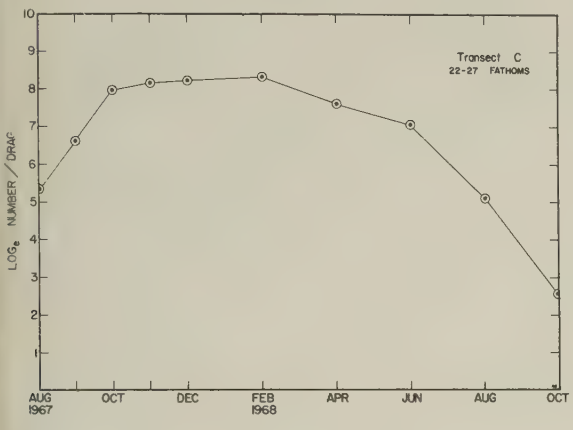

FIGURE 4.—Catch curve for transect C, 22 to 27 fm. Data are for the 1967 year class.

The February to October segment was treated linearly to estimate monthly mortality. Results were $i = -0.231$ and $a = 21\%$ (see Table 1). Mortality rates for the dome (October to February) were not calculated.

Although these rates are similar to those in Table 3, enough discrepancy exists to indicate more than one year class might have been included in Table 3 data, causing some differences in mortality rates. This does not preclude mortality rate differences on the grounds.

The major calico scallop predator appears to be the starfish (*Asterias*) which is often taken in large numbers in dredge tows (Figure 5) and has been seen by submarine observers feeding on scallops (Figure 6). Rays and skates may feed on calico scallops (Struhsaker, 1969) and puffers (*Sphoeroides*) have been taken with numerous small (2 to 5 mm) scallops in their stomachs. Other predators are not known.

Little is known about those environmental factors affecting scallops though water temperature is considered most important. Past explorations have shown evidence of occasional mass mortalities that may have been due to temperature fluctuation. Dickie and Medcof (1963) reported that mass mortalities often occur in sea scallops when water temperatures fluctuate rapidly. Further, temperature changes may indirectly cause death through dehabilitation, thereby rendering scallops highly susceptible to predation.

DISTRIBUTION AND ABUNDANCE

Calico scallops occurred in 13 to 37 fm with greatest concentrations between 19 and 30 fm (Figure 1).

Depth distributional differences north and south of the Cape were noted (Figure 7). Scallops south of the Cape were generally found shallower than north of the Cape. The reasons for this are unknown though the thermal structure may be different in these areas. Also the shelf is narrower with a steeper gradient south of the Cape, and available habitat is restricted. Optimum bottom may occur 4 to 5 fm shallower in that area.

A slight seasonal change in depth distribution occurred in both areas. Scallops were in slightly deeper water in winter than summer, this difference being less noticeable north of the Cape.

Yearly differences in bed distribution were noted during the survey. In 1967 beds were primarily located between 19 and 30 fm; however, in the fall of 1968 very few scallops occurred in that depth range and a developing bed was found at lat 29°10′ N in 15 to 17 fm. This bed extended northward beyond lat 29°25′ N.

Scallops were usually found in north-south windrows several hundred yards to a quarter mile long. Bed size varies but without a means

FIGURE 5.—Dredge catch of starfish aboard the RV *Oregon* taken during an exploratory fishing survey on the Cape Kennedy grounds.

of underwater observation there is no way of mapping beds definitively. A remote-controlled underwater assessment vehicle (RUFAS) has since been used to survey the Cape Kennedy grounds and preliminary data analysis verifies our findings (Cummins et al., see footnote 5).

Major abundance occurred at slightly different depth ranges north and south of the Cape (Figure 8). Seasonal changes, while not noticeable in these areas, did occur at some depth ranges. Abundance was highest in 21 to 23 fm south of the Cape and 24 to 27 fm north of the Cape. By August 1968, scallops were gone south of the Cape. North of the Cape the population diminished through October 1968 and had practically disappeared by December.

YIELD

Yield is presented as pounds of meat per drag. Preliminary calico scallop investigations had used bushels per drag as a yield criterion, but owing to differences in size, barnacle encrustation on the shell, and changes in meat condition, the meat yield per bushel was highly variable.

Variation in bushel yield due to size differences is obvious, but the effect of barnacle encrustation is more subtle. The sedentary nature

FIGURE 6.—Starfish (*Asterias*) observed attacking calico scallops during a submarine survey off Cape Kennedy in September 1969.

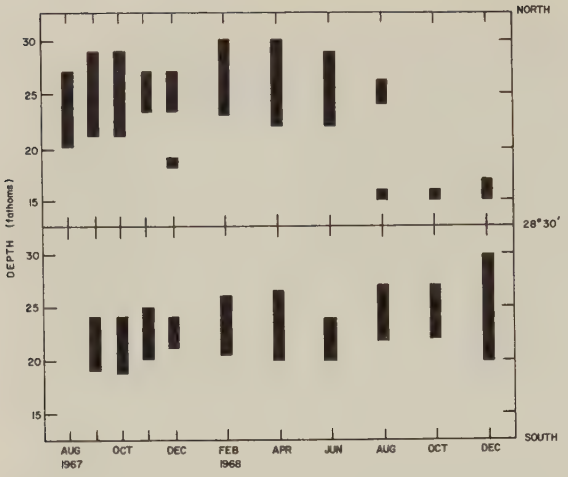

FIGURE 7.—Monthly depth distributions north and south of Cape Kennedy.

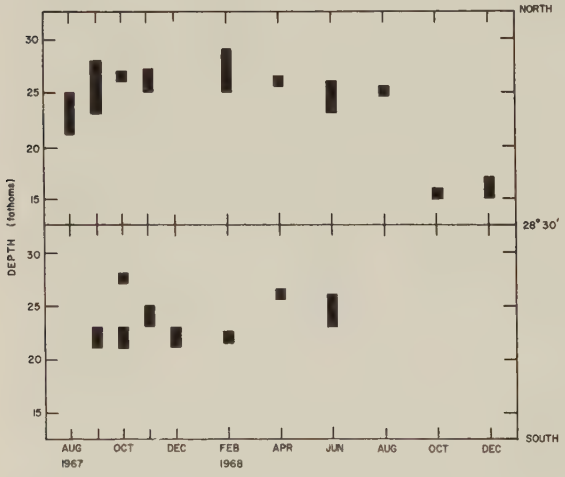

FIGURE 8.—Depths at which maximum abundance occurred north and south of Cape Kennedy.

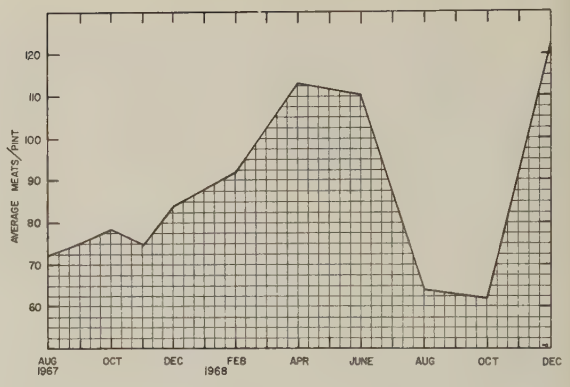

FIGURE 9.—Average number of scallop meats per pint by month during the 1967-68 Florida east coast survey.

of scallops enables a gradual buildup of barnacles on the shell which decreases the number of scallops per bushel. This leads to an overestimate of meat yield per bushel.

Meat condition was found to fluctuate seasonally. From December to April meat counts increased rapidly (Figure 9) owing to increased flaccidity resulting from physiological changes associated with spawning. Though most scallops are generally large (50 to 60 mm) at spawning, the meat yield per bushel is much lower than during the fall when scallops are smaller.

These conditions caused us to discard number of bushels and use total pounds of meat per drag as a more meaningful measurement of yield.

Meat count per pint is an excellent index of fishing productivity when used in conjunction with total pounds. A wide seasonal variation in meat count was caused by differences in meat condition and scallop size (Figure 9). From August to December the meat count per pint stabilized at 70 to 80. During December through June the count increased due to deterioration of meat firmness and/or contribution by a nonspawning population remnant.

The fall increase in meat count per pint is due to increasingly large numbers of small scallops entering the fishery from the shallow bed on transect C.

Commercially significant yields were taken between September and February with a maximum in October (Figure 10). Yield rapidly decreased after February because of spawning. Yield in the fall of 1968 was appreciably lower than in 1967 owing to the failure of the survey to locate quantities of scallop. The population in 15 to 17 fm at lat 29°10′ N, spawned in 1968, was not found in any abundance during the Oc-

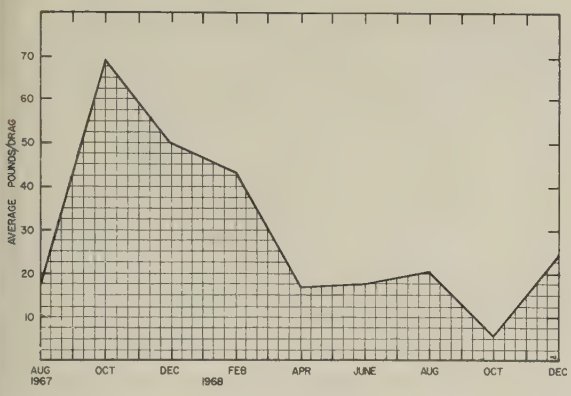

FIGURE 10.—Average number of pounds of scallop meats per 30-min drag by month during the 1967-68 Florida east coast survey.

tober or December cruises, indicating spawning occurred much later in 1968 than in 1967.

DISCUSSION

Reproduction in calico scallops is related to age rather than size. It is probably triggered by water temperature as Loosanoff and Davis (1950) have shown that raising the ambient water temperature induces spawning in some bivalves. Since spawning generally occurs in the spring, rising temperatures would be expected to be the initiating mechanism.

Maturation, taking 7 to 9 months beginning in late summer, is easily detected in the field by coloration changes in the ovaries. Resting ovaries are whitish-yellow but as maturation progresses their color changes through various stages of deepening yellow-oranges to a reddish-orange at the ripe condition. Ripe gonads have been collected from a wide size range indicating that the minimum age of sexual maturity is quite low. Data indicate maturation does not begin simultaneously in all scallops within even the same bed. This presumably accounts for the 2- to 3-month differential in maturation time.

Spawning begins in late February or early March and continues to June. In some areas the season is protracted since small scallops are caught throughout most of the year. This is probably due to variation in sexual maturation rate, growth rates, and water temperature. The lowest frequency of small scallops occurred in February indicating decreasing temperatures in the fall terminate any protracted spawning.

Protracted spawning during one season conceivably leads to protraction during the next. Since growth is related to temperature, scallops spawned early in spring have a longer growing season than do those spawned later in the year. Therefore, the older individuals in a year class would undoubtedly be larger at spawning than the younger individuals. Further, maturation is probably controlled by hormones which in turn are influenced by some environmental factor such as water temperature. This interaction between age, environment, and reproduction needs clarification.

Growth curves through all sizes ranges cannot be calculated from the survey data since the earliest stages are omitted. The curve is sigmoid from seed size to senility, rapidly increasing from 5 to 50 mm. Then the rate decreases through senility with maximum size about 80 mm.

Data from 1967 and 1968 indicate only one year class is present in spring but two year classes are present from early summer through winter. Therefore, the maximum age reached by calico scallops must not be greater than 24 months and averages 18 to 20 months.

Mortality rates computed from the survey data support a 2-year maximum life span. Post-spawning mortality averages 23% per month. At that rate only 20% of the year class would remain by early fall. Assuming that some mortality occurs during the pre-spawning period, the remaining population in 18 to 20 months would be exceedingly small.

Scallops are not randomly distributed but form north-south windrow configurations. These configurations, established at spat set, are heavily influenced by currents. Olsen (1955) indicates that windrow-like configurations result from strong tides, their orientation running lengthwise to the tide. He also shows strong linear configurations result in eddy systems.

This latter situation is analogous to that off eastern Florida. Little is known about substrate preference. Some beds offering optimum conditions may be perpetuated for several years, but competition for space between adults and spat must minimize such occurrences. If spat are unable to compete effectively for substrate, the presence of live adult scallops on the bed may prevent spat set. This question needs to be answered in the near future.

Yearly distribution and abundance depend on both spawning success and spat set. Commercial size and abundance are generally reached by early fall, usually in October. Fishing remains optimal until February when catch rates and yield decrease. This is due to spawning and associated factors such as increased mortality and meat deterioration.

In October 1967 scallops averaged about 45 mm and yielded 75 to 80 meats per pint. During October the survey produced an average catch of 70 lb. of meats per 30-min drag as compared with 20 lb/drag in April.

A fall and early winter fishery is expected to be most productive. Fishing is not recommended during spring because catch rates and yield are generally low and a spring fishery could have an adverse effect on the spawning population.

The nonrandom distribution and variable size of beds make it difficult to estimate the standing crop from dredge surveys. Scallops were found over approximately 285 square miles of bottom during the survey but less than 14% (37 miles) was covered by the dredge. About 6,500,000 scallops were caught during the survey. Scallops are not randomly distributed on the grounds but occur in beds where very high densities are often reached. Relatively few individuals occur between beds. Beds are difficult to measure but some were estimated to be several hundred yards in width and over a half mile in length.

Photographs taken during the *Aluminaut* dive show that densities of five scallops per square foot occur on some beds (Taylor, 1967). A minimum of 285 square miles of scallop bottom was found during the survey and varied densities occurred over that area. If 10% (28 square miles) supported densities approximating those found by the *Aluminaut*, then an estimated standing crop of 3,892,000,000 scallops existed in 1967-68. This figure is derived from the following: 28×27.8 million (no. $ft^2/mile^2$) $\times 5$. That population could easily support 10 boats fishing at a rate of 1,500 lb/day. At an average of 70 scallops per pound, 10 boats would take a total of 31,500,000 scallops per month at the above rate. This is approximately 1% of the estimated standing crop.

Recent explorations with RUFAS indicate that densities estimated from the *Aluminaut* cruise may be ultraconservative (Cummins et al., see footnote 5). Films from the RUFAS survey indicate that scallops may reach densities as high as 10 or more per square foot providing a standing crop is considerably above that given previously. Findings from the RUFAS survey will be published in the near future by personnel from the Exploratory Fishing Station in Brunswick, Ga.

Yearly variations in distribution and abundance may at first glance be discouraging to a fishery. However, the remarkable opportunity to predict each fall fishery exists because distributional and abundance patterns are established in spring or early summer—perhaps as early as May or June. Population assessment at that time would provide estimates on the standing crop and determine the success of a fall fishery some 4 or 5 months prior to its onset. The authors tested this hypothesis in the spring and summer of 1970 using the RUFAS vehicle.

SUMMARY AND CONCLUSIONS

The survey data show that calico scallops have a short life span of 18 to 24 months. Spawning occurs after a 7- to 9-month sexual maturation period in early spring. Some protracted spawning was noted for localized areas. Although growth rates were not determined for sizes smaller than about 5 mm, the estimated growth curve between 5 mm and senility (75 to 80 mm) is sigmoid, rapidly increasing to about 50 mm and then decreasing to death.

Monthly mortalities for December to October average approximately 20%. Mortality curves were generally curvilinear after spawning, indicating a rapid post-spawning dieoff.

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EFFECTS OF DELAYED INITIAL FEEDING ON LARVAE OF THE GRUNION, *Leuresthes tenuis* (AYRES)

ROBERT C. MAY[1]

ABSTRACT

The initial feeding of newly hatched larvae of the grunion, *Leuresthes tenuis* (Ayres), was delayed for various periods of time under laboratory conditions at 18° C. Unfed larvae did not develop morphologically beyond the stage reached at the time of yolk absorption, about 4 days after hatching, although some survived as long as 3 weeks. Regardless of how long initial feeding was delayed, 80% or more of previously unfed larvae began feeding when food was made available to them, and at least 40% of the larvae alive when food was offered were able to survive to the end of a 20-day experiment. Some larvae feeding for the first time after 1 to 2 weeks without food, died after gorging themselves with *Artemia* nauplii. When food was offered to starved larvae, growth began and generally proceeded at about the same rate as in larvae fed from day 1, although there was some indication that a few days' delay in initial feeding increased the conversion efficiency of grunion larvae feeding on *Artemia* nauplii. Catabolism of fat provided most of the energy for metabolic processes during starvation. The condition factors and carbon/nitrogen ratios of unfed larvae were below those of fed larvae; condition factor seemed to be the better index of nutritional state. Grunion larvae probably do not experience high mortality at sea due to starvation, nor do they exhibit a classical "critical period" at the time of yolk absorption.

Most marine fishes pass through a free-swimming larvae stage, and it is well documented that survival through this stage is very low, generally being much less than 0.1% (e.g., Sette, 1943; Ahlstrom, 1954; Pearcy, 1962; Iizuka, 1966). The rate of survival through the larval stage is probably the most important factor determining the strength of year classes (Beverton, 1962; Gulland, 1965). Hjort (1914, 1926) advanced the hypothesis that larval survival was drastically affected by the abundance of food at the time the yolk was completely absorbed, and that poor year classes resulted when insufficient food was available to larvae at this "critical period." As Marr (1956) pointed out, Hjort's "critical period" concept has had a profound effect upon the thinking of fishery biologists.

Increased larval mortality at the time of yolk absorption has, however, proved difficult to demonstrate in nature. Marr (1956) concluded that the published evidence did not establish the existence of such increased mortality at sea; even in the light of more recent field data (Farris, 1961; Pearcy, 1962; Stevenson, 1962; Iizuka, 1966; Karlovac, 1967), it is difficult to decide from survival curves whether increased mortality at yolk absorption does in fact occur in nature. It has proved equally difficult to demonstrate that starvation is a major cause of larval mortality in the sea. Wild larvae found with empty guts (Lebour, 1920; Bowers and Williamson, 1951; Arthur, 1956; Bhattacharyya, 1957; Berner, 1959) may indicate imminent death by starvation or may reflect artifacts such as defecation or selective capture by plankton nets (Blaxter, 1965, 1969). Reports of apparently emaciated larvae, sometimes caught in regions where food is scarce (Soleim, 1942; Arthur, 1956; Shelbourne, 1957; Nakai, 1962; Hempel and Blaxter, 1963; Nakai et al., 1969), are suggestive but inconclusive (Marr, 1956; Blaxter, 1965, 1969). Field data thus indicate the possibility of high larval mortality due to starvation after the yolk has been absorbed but have not demonstrated its existence conclusively

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or determined its significance for year-class strength.

The response of larvae to food deprivation in the laboratory may provide badly needed evidence of how susceptible they are to starvation at sea. There have, however, been few attempts to determine experimentally the effects of delayed initial feeding on the larvae of marine fishes. Fabre-Domergue and Biétrix, the two pioneers of marine fish culture who coined the term "critical period," which Hjort later adopted, believed that feeding before the yolk supply was exhausted was essential to assure larval survival in laboratory rearing attempts. They stated that among larvae which received food prior to yolk absorption, ". . .*la phase que nous avions nommée période critique post-larvaire n'existe pas*" (Fabre-Domergue and Biétrix, 1898: 468). These authors went on to say that larvae which did not receive food early would subsequently become weak and unable to capture food and would exhibit considerable, if not total, mortality (Fabre-Domergue and Biétrix, 1898). The importance of early feeding for marine fish larvae was not further investigated in the laboratory until Blaxter and Hempel (1963) studied the effects of delayed initial feeding on the behavior of larval herring, *Clupea harengus* L. By feeding larvae after successively longer times without food, Blaxter and Hempel determined the time beyond which the larvae failed to exhibit feeding movements when supplied with food, a time they called the "point of no return." This point came 5 to 9 days after complete yolk absorption, at temperatures of 12° to 8° C, much later than the statements of Fabre-Domergue and Biétrix would have led one to expect. Recently, Lasker et al. (1970) observed the mortality of larvae of the northern anchovy, *Engraulis mordax* Girard, which had been fed at progressively later times after hatching. At temperatures of 15° to 22° C, larvae for which initial feeding had been delayed until 2.5 days after complete yolk absorption, showed the same pattern of mortality as groups of starved controls, while larvae receiving food 1.5 days after yolk absorption exhibited good survival—a phenomenon which these authors termed "irreversible starvation."

The purpose of the present study was to investigate in detail the changes which take place in starving larvae and in larvae whose initial feeding is delayed for various lengths of time, and thus to bring more evidence to bear upon the perennial questions of how susceptible larval fishes are to food deprivation and whether they do pass through a "critical period" at the time of yolk absorption. This study also sought to broaden the range of our knowledge of larval fish ecology by utilizing a species belonging to a group other than the Clupeiformes or Pleuronectiformes, or which nearly all of our information has hitherto been based. The fish chosen for study was the grunion, *Leuresthes tenuis* (Ayres), a member of the family Atherinidae. Atherinids produce rather large demersal eggs (Breder and Rosen, 1966), and the well-developed larvae which hatch provide an interesting contrast with flatfish and clupeoid larvae. Specifically, these experiments were designed to determine the effects of delayed initial feeding on mortality, on growth, and on the ability of grunion larvae to begin feeding and to utilize ingested food, and to ascertain what changes in the morphology and chemical composition of the larval body occur during starvation.

MATERIALS AND METHODS

SOURCE OF EGGS; HATCHING

The grunion is best known for its unusual habit of spawning on the sandy beaches of southern California and northern Baja California (Thompson and Thompson, 1919; Walker, 1952; Breder and Rosen, 1966). The eggs are deposited in the sand at night at certain times in the tidal cycle and are washed free some days later by a succeeding high tide, at which time the larvae hatch if the developmental period has been sufficiently long. The spawning season extends from late February or early March to late August or early September, with spawning intensity reaching a peak in April and May (Walker, 1952). During this time it is relatively easy to collect grunion eggs, which therefore provide

convenient material for the study of embryonic and larval development.

Eggs for the present study were collected during a spawning run on the night of March 24, 1970, at the beach in front of Scripps Institution of Oceanography, La Jolla, Calif. The eggs from a running ripe female were expressed into a small plastic container and artificially fertilized by adding milt from one male and a small amount of seawater; after approximately 1 min the water was decanted and sperm removed with several washes of fresh seawater. At the National Marine Fisheries Service Fishery-Oceanography Center in La Jolla, developing eggs were dispersed in a layer of washed, slightly moist beach sand approximately 1 cm deep at the bottom of rectangular plastic containers ($16 \times 12 \times 11$ cm), and a paper towel moistened with seawater was placed on the surface of the sand. The tops of the containers were covered with aluminum foil. This incubation procedure, essentially the same as one described by Morris (1956), kept the eggs moist and produced good hatching when excess water (which quickly brought on anoxic conditions) was avoided. The containers were placed in a water bath held at 20° ± 1° C by manually mixing water from the warm and cold seawater systems of the Fishery-Oceanography Center (see Lasker and Vlymen, 1969). The day before hatching, the temperature of the water bath was lowered to 18° C over a period of 3 hr.

On April 3, 1970, after 10 days of incubation (a common incubation time in nature, according to Walker, 1952), hatching was induced by adding filtered seawater at 18° C to the incubation containers and agitating the water and sand by drawing water rapidly in and out of a pipette. Hatched larvae were immediately transferred via pipette (4-mm bore) to 18° C water in rearing containers. In this paper the day of hatching will be referred to as day 0, the day after as day 1, and so forth.

DESIGN OF EXPERIMENT

A total of 20 rearing containers was set up, each with approximately 50 newly hatched grunion larvae. Seven containers (#1-7) were used to determine the effect of delayed initial feeding on mortality and growth; in six containers in this group, feeding was begun at progressively later times at 3-day intervals beginning on day 1—i.e., on days 1, 4, 7, 10, 13, and 16—while the larvae in one container (#7) were not fed and served as a control. On the 20th day after hatching, all surviving larvae which had received food in this series were collected, and the length, weight, and chemical composition of the larvae were determined. Seven of the other containers (#8-14) were fed daily beginning on day 1, and six (#15-20) were given no food; these containers, referred to as "supply containers" in what follows, supplied fed and unfed larvae for measurements of length, weight, and chemical composition and also for experiments on feeding and growth. On the same days when feeding was begun in a new container in the delayed feeding series (containers #1-7), larvae from both the "fed" and the "unfed" supply containers were used to determine the incidence of feeding and to begin quantitative feeding experiments.

PHYSICAL CONDITIONS

Water of approximately 33 ‰ salinity was taken from the seawater system of the Fishery-Oceanography Center. The larval fish containers were originally filled with HA Millipore-filtered seawater, and at weekly intervals filtered water was added to the fed containers in order to replace water removed with uneaten food and fecal matter (see below), the volume added usually being between 1 and 2 liters. The temperature in the water bath was kept at 18° ± 1° C, and temperatures in the larval containers were within 0.5° C of the bath temperature. Eighteen degrees is the midpoint of the 14°-22° C range of water temperatures which occurs off La Jolla during the spawning season of the grunion (Reid et al., 1958). Banks of two 40-watt "daylight" fluorescent lamps positioned 76 cm above the surface of the water illuminated the containers for 12 hr each day. The lights were timed to go on after sunrise, so that diffuse light entering through windows increased

slowly in intensity as the sun rose and no abrupt dark-light transition was imposed upon the larvae.

CONTAINERS

The containers used for rearing larvae in this study were the same as those described by Lasker et al., 1970), being circular (35 cm diameter, 14 cm deep) and made of the plastic alloy Kydex.[2] These containers have nonglossy black surfaces and hold 10 liters of seawater. For feeding studies with individual larvae, smaller Kydex containers were used (10.5 cm diameter, 4.2 cm deep) which held 300 ml of water. Both large and small containers were covered with clear plexiglass tops to reduce evaporation and keep out dust particles.

FEEDING

Nauplii of the brine shrimp, *Artemia salina*, were used exclusively as a food source. *Artemia* nauplii have proven to be poor food for larval clupeids but excellent for several other types of larvae (May, in press), and they appear to satisfy the nutritional requirements of larval grunion. Nauplii were obtained by hatching San Francisco brine shrimp eggs in trays modeled after those described by Riley (1966). Two trays were used, which allowed harvesting of trays on alternate days with a time lapse of 48 hr between inoculation of eggs and harvesting of nauplii. The water in the trays was kept at about 20° C. Nauplii were rinsed in filtered seawater and added to rearing containers shortly after the lights went on each morning, and more were added during the day if the concentration dropped low enough to prevent *ad libitum* feeding. If any uneaten nauplii remained from the previous day, as many as possible were removed by pipette before the morning addition of new nauplii. Fecal matter was siphoned daily from the bottoms of the "fed" containers.

[2] Kydex is manufactured by Rohm and Haas, Philadelphia, Penn. Use of trade name does not imply endorsement of the product.

QUANTITATIVE FEEDING STUDIES

At 3-day intervals beginning on day 1, 6-day quantitative feeding experiments were begun to measure the food consumption and growth of previously fed and unfed larvae. Larvae were transferred individually, from both the "fed" and the "unfed" supply containers, to small (300 ml) containers late in the afternoon on the day before the beginning of the feeding experiment. Three larvae from a "fed" container and three from an "unfed" container were used in each feeding experiment; individual larvae were kept in separate containers during the feeding study.

On the morning following transfer to the feeding containers, and for six mornings thereafter, a known number of *Artemia* nauplii was counted out with a pipette under a dissecting microscope and added to each container. Shortly before the lights went off at the end of the day, the grunion larvae were transferred by pipette to new containers, and the uneaten *Artemia* in the old containers were concentrated on a nylon mesh, preserved in Formalin and later counted. The difference between the number of nauplii added in the morning and the number left at the end of the day gave the number eaten by a larva. At the start of the series of feeding experiments, on day 1, 100 nauplii were added to each experimental container; when larvae consumed 70% or more of the nauplii offered, the number offered the following day was increased by 50 nauplii. On the morning following the final (6th) day of feeding, the experimental larvae were collected and analyzed as described below. The weight of a larva at the start of the feeding experiment, estimated from the mean weight of 10 larvae sampled at that time, was subtracted from the weight of the experimental larva at the end of the feeding period to yield its gain in dry weight.

In order to determine the weight of the ingested material, the weight of a single *Artemia* nauplius was estimated by making several weighings on an electrobalance[3] of groups of 5 to 20 nauplii, collected at the same interval after inoculation of eggs as the nauplii used in the feeding study. The nauplii were rinsed with distilled water and dried to constant weight at

60° C before weighing. The mean weight per nauplius was 1.64 μg, almost identical to Paffenhöfer's (1967) value of 1.65 μg per newly hatched *Artemia* nauplius. Where the larvae had yolk sacs at the beginning of the feeding period, the mean weight of the yolk masses dissected off the 10 larvae sampled at the start of the experiment (the dissection technique is described below) was added to the weight of the nauplii consumed to yield the total dry weight of the consumed material.

The growth and food consumption of individual larvae during the feeding period were thus known and allowed calculation of the efficiency of food conversion:

$$\text{percent conversion efficiency} = \frac{\text{dry weight gained}}{\text{dry weight consumed}} \times 100.$$

INCIDENCE OF FEEDING

The percentage of larvae which fed after progressively longer times without food, termed here the incidence of feeding, was determined in separate experiments in 10-liter containers. Approximately 25 larvae were transferred from one of the "unfed" supply containers to a 10-liter container with filtered seawater late in the afternoon preceding the experiment, and on the following morning large numbers of *Artemia* nauplii were introduced into the container. One hour later, the anesthetic MS-222 (tricaine methanesulfonate) was added to the container to a concentration of 132 mg/liter, and the anesthetized larvae were examined under a dissecting microscope for the presence of an orange-colored gut indicative of feeding on *Artemia* nauplii. Simultaneously, the incidence of feeding among larvae from one of the "fed" supply containers was determined in the same way, to serve as a control with which hitherto unfed larvae could be compared. Experiments of this sort were conducted on the same days on which food intake and conversion experiments were started, beginning with day 4. Owing to mortality from starvation, the numbers of larvae available in the "unfed" containers dwindled so that only 13 larvae were available for the feeding incidence experiment on day 13 and 4 on day 16. By day 16, body pigmentation had developed in larvae from the "fed" containers to such an extent that feeding incidence could not be assessed by examining the coloration of the gut, and no value was obtained for previously fed larvae on this day.

Anesthesis stimulated peristalsis in grunion larvae, as Blaxter (1965) observed in larval herring, but the procedure in the present experiment was rapid enough that at most only the contents of the rectum were being extruded during examination and all larvae which had in fact fed were recorded as such. It should be pointed out that, unlike the straight, tubelike gut of clupeid larvae, the gut of larval grunion is already differentiated at hatching into three more or less distinct portions, the last of which, the rectum, is separated from the rest of the gut by an "ileorectal valve" (Al-Hussaini, 1947) which inhibits rapid defecation of material not in the rectum.

MORTALITY

Dead larvae were removed from the containers each morning by pipette. A larva was considered dead when its brain had become opaque and it did not respond to water current or to tactile stimulation. Dead larvae were routinely examined with a dissecting microscope.

SAMPLING PROCEDURE AND ANALYSIS OF LARVAE

Larvae were collected by pipette, and their lengths measured with an ocular micrometer from snout to tip of notochord, or, after upward flexion of the tip of the notochord had taken place, to the posterior edge of the hypural elements (standard length). Only free-swimming larvae were sampled, although in "unfed" containers these became increasingly rare toward the end of the experiment. Sampled larvae were rinsed quickly in distilled water and placed on glass microscope slides. Since larvae which were sampled on days 1 and 4 still possessed yolk sacs, they were preserved in 3%

[3] Cahn Instrument Company, Paramount, Calif.

Formalin (in 50% seawater). Within 1 week of sampling they were rinsed in distilled water and their yolk dissected off, separated larvae and yolk masses being placed on microscope slides. The samples on slides were dried to constant weight at 60° C and weighed to the nearest microgram on an electrobalance. In agreement with the results of Blaxter and Hempel (1966), no effect of Formalin on the dry weight of larvae was found, nor was there a significant effect of Formalin on the dry weight of yolk masses (this was tested in a previous experiment by comparing dry weights of yolk dissected from preserved larvae with yolk dissected from frozen larvae or collected in preweighed capillary tubes).

Larvae sampled from the supply containers at 3-day intervals, and those collected from the delayed-feeding series on day 20, were analyzed for their ash, carbon, hydrogen, and nitrogen content. Percent ash was determined by weighing separately three randomly chosen larvae from each sample before and after combustion at 500° to 520° C. In the case of larvae fed from day 16 and sampled on day 20, only one larva was available for the ash determination. During combustion, larvae were held on tarred pieces of precombusted aluminum foil, and weighings were made on an electrobalance. The remaining larvae in each sample were ground into fine particles with an agate mortar and pestle, and two aliquots of this material from each sample were analyzed for carbon, hydrogen, and nitrogen content with a Model 185 carbon-hydrogen-nitrogen analyzer.[4] The number of replicates was limited by the amount of material available, but variation between replicate determinations was small, and means calculated from the replicates were accepted as the ash, C, H, and N values for the sample. This approach to the chemical analysis of larvae was chosen because it allowed determination of C/N ratios, estimation of protein and fat content, and calculation of caloric content (see Results section).

Larvae which had been used in the feeding incidence experiments described above were preserved in 3% Formalin and later cleared in KOH and stained with Alizarin Red-S, the standard stain for bone, to allow comparison of ossification in fed and unfed larvae.

RESULTS

BEHAVIOR

Newly hatched grunion larvae have functional eyes and jaws and are extremely active (Thompson and Thompson, 1919; David, 1939). Grunion larvae which received food in the present experiment remained very active as they grew, and some schooling behavior was noted as early as day 6. Of more immediate concern was the behavior of starved larvae. On day 7 it was noted that unfed larvae were much easier to catch with a pipette than fed larvae. As the period of starvation lengthened, larval activity declined and the number of larvae remaining quiescent on the bottom increased. Near the end of the experiment, no starved larvae were swimming freely above the bottom, and their activity consisted in occasional erratic movements, followed by long quiescent periods.

SURVIVAL

Figure 1 shows the survival to day 20 of larvae which were fed at various times after

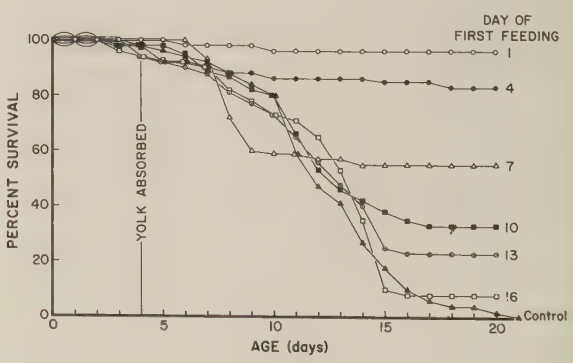

FIGURE 1.—Survival curves for larvae with different times of initial feeding, at 18° C. The number at the end of each curve indicates the day of initial feeding. The control group was given no food during the experiment.

[4] Hewlett Packard Corporation, Palo Alto, Calif.

hatching and a starved control. Yolk was completely used up in unfed larvae by day 4, and on this day only a minute amount was left in fed larvae. The survival curve for unfed larvae passed the 50% line between days 11 and 12, roughly the same as the starvation time given by Hubbs (1965) for larval grunion at 18° C. The starved control larvae were all dead by day 21. There is a direct relationship between percent survival of original larvae to day 20 and the day of first feeding (Table 1). The number

TABLE 1.—Survival to day 20 of larvae with different times of initial feeding.

Day of first feeding	Original number of larvae	Number of larvae alive when food first offered	Number of larvae alive on day 20	Percent survival to day 20	
				Original larvae	Larvae alive when food first offered
1	51	51	49	96.1	96.1
4	52	51	43	82.7	84.3
7	53	49	29	54.7	59.2
10	55	44	18	32.7	40.9
13	48	23	11	22.9	47.8
16	51	4	4	7.8	100.0

of larvae which survived to day 20, expressed as a percentage of those which were alive when food was first offered, is also listed in Table 1. This figure never dropped below 40%, and all of the previously unfed larvae alive in container #6 on day 16 (i.e., four larvae) began feeding when food was supplied and survived to day 20, and their general appearance and behavior indicated that they would easily have survived longer, had the experiment been prolonged.

In the group of larvae which was fed for the first time on day 7, 11 larvae were found dead on the morning following the day of first feeding; 9 dead larvae had food in their guts and 5 of these had guts which were bright orange and so stuffed with *Artemia* nauplii that the abdomen was noticeably distended. In the group fed initially on day 10, all eight larvae found dead the next morning had food in their guts, and four of these had bright orange, packed guts. On day 14, only one out of four dead larvae, found in the container which had been fed for the first time on the previous day, had food in its gut and showed the orange and bulging abdomen noted in dead larvae from the previous two groups.

GROWTH

The increase in length and dry weight of larvae sampled from the "fed" supply containers is presented in Table 2. The rate of growth was higher from day 16 on; variability likewise increased after this time, an example of the "growth depensation" which is commonly found in growing fish (Ricker, 1958). On day 7, the hypural elements were beginning to form along the posterior ventral margin of the notochord, and on day 10 the tip of the notochord was beginning its upward flexion. The greatest increase in length occurred between days 1 and 4, and owing to the upturning notochord the mean length actually decreased between days 13 and 16 (Table 2). On day 4, only the cleithrum and a very few cranial and branchial elements were ossified, but by day 10 about half of the vertebrae (the anterior ones) and some of the caudal rays were beginning to take up alizarin, and by day 16 all vertebrae and hypural elements were ossified.

TABLE 2.—Length and weight of fed and unfed larvae. $\bar{x}$ = mean, *SD* = standard deviation, n = number of larvae measured.

Age (days)	Length (mm)						Dry weight (mg)					
	Fed			Unfed			Fed			Unfed		
	$\bar{x}$	*SD*	n	$\bar{x}$	*SD*	n	$\bar{x}$	*SD*	n	$\bar{x}$	*SD*	n
1	--	--	--	8.96	0.17	9	--	--	--	0.362	0.021	9
4	9.64	0.34	10	9.31	0.20	10	0.428	0.038	10	0.386	0.030	10
7	10.70	0.59	10	9.27	0.12	10	0.771	0.130	10	0.409	0.015	9
10	11.66	0.28	10	9.11	0.23	10	1.027	0.100	10	0.355	0.032	10
13	12.28	0.34	10	8.98	0.19	10	1.340	0.183	10	0.311	0.013	10
16	12.22	0.60	10	8.78	0.19	10	1.517	0.361	10	0.266	0.018	10
19	13.53	0.58	10	--	--	--	2.433	0.458	10	--	--	--
25	15.12	0.45	10	--	--	--	3.804	0.464	10	--	--	--

Starved larvae exhibited a slow decline in dry weight after yolk absorption, with little variability between larvae (Table 2). Although rudiments of the hypural elements were just discernible in starved larvae on day 7, their notochords never showed evidence of upward flexion, even as late as day 16. On day 16, ossification in starved larvae was comparable to that in fed larvae from day 4 or day 7, with only the cleithrum and a few elements of the cranium and visceral skeleton taking up alizarin.

The lengths and weights of 20-day-old larvae from the delayed feeding series (containers #1-6) are listed in Table 3, and in Figure 2 the

TABLE 3.—Length and weight of 20-day-old larvae with different times of initial feeding. $\bar{x}$ = mean, SD = standard deviation, n = number of larvae measured.

Day of initial feeding	Length (mm)			Dry weight (mg)		
	$\bar{x}$	SD	n	$\bar{x}$	SD	n
1	14.24	0.61	20	2.702	0.420	20
4	14.03	0.36	20	2.513	0.193	20
7	12.38	0.31	20	1.638	0.148	20
10	11.10	0.38	18	0.995	0.086	18
13	9.87	0.51	11	0.561	0.137	11
16	9.82	0.35	4	0.436	0.036	4

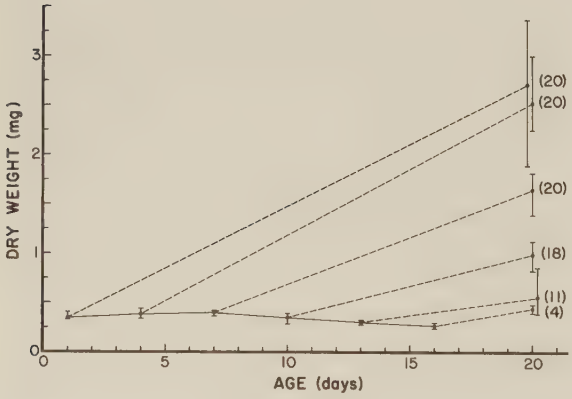

FIGURE 2.—Dry weights of 20-day-old larvae with different times of initial feeding. Means and ranges are plotted, and the number of 20-day-old larvae measured in each group is given in parentheses. Dashed lines connect mean weights at day 20 with the mean weights of unfed larvae on the days when feeding was initiated.

weights are plotted and connected by dotted lines to the weights of starved larvae on the days when food was first offered. As expected, the later the initial feeding, the lower the mean weight on day 20, although the range of weights of larvae fed from day 4 falls within that of larvae fed from day 1, and larvae fed from days 13 and 16 likewise overlap (Figure 2). The rate of gain in weight decreases with delay of initial feeding, as indicated by the decreasing slopes of the dotted lines in Figure 2, but these rates are similar to those of larvae feeding for comparable lengths of time from day 1 (e.g., the weight gain of fed larvae between days 1 and 10 is about the same as that of larvae fed initially on day 10 and sampled on day 20). Larvae fed from days 1, 4, and 7 had completed notochordal flexion by day 20, and those fed from day 10 were at an intermediate stage of flexion at this time; in those fed from days 13 and 16, the process of flexion had not yet begun by day 20. Larvae from both the fed series and the delayed-feeding series indicated that notochordal flexion did not begin until a length of about 11 mm and a dry weight of about 1 mg had been reached.

Condition factors have been used in the past to assess the nutritional state of fish larvae (Hempel and Blaxter, 1963; Blaxter, 1965) and were calculated for each sample in the present experiment as

$$\frac{\text{(mean dry weight, mg)}}{\text{(mean standard length, mm)}} \times 10^3$$

Figure 3A shows that after day 4, the condition factors for fed larvae increased until the end of the experiment on day 25, while those for starved larvae, after showing a slight rise on day 7, decreased until the final sampling on day 16. As shown in Figure 4A, the condition factors of 20-day-old larvae decreased in groups for which initial feeding had been delayed 7 days or more, with larvae fed from day 16 showing a condition factor between those of starved larvae 10 and 13 days old.

FEEDING INCIDENCE

On day 4, larvae which had been offered food since day 1 showed a higher feeding incidence (88%) than those which were given food for

the first time on this day (35%)—Table 4. However, on days 7, 10, and 13 the feeding incidence was similar (between 80 and 96%) in larvae which had fed and those which had not fed prior to the test. On day 16, the darkly pigmented abdomen of previously fed larvae made it impossible to determine their feeding incidence, but all of those larvae tested which had received no food prior to day 16 did consume food on this day. The failure of previously fed larvae to show a feeding incidence of 100% in these experiments probably reflects the stress associated with transfer between containers.

TABLE 4.—Feeding incidence of previously fed and unfed larvae. Larvae were exposed to *Artemia* nauplii for 1 hr, after which they were examined for evidence of feeding.

Age (days)	Larvae previously fed			Larvae previously unfed		
	Number of larvae feeding	Number of larvae not feeding	Percent feeding	Number of larvae feeding	Number of larvae not feeding	Percent feeding
4	22	3	88.0	9	17	34.6
7	21	2	91.3	23	1	95.8
10	22	2	91.7	24	1	96.0
13	20	4	83.3	8	2	80.0
16	--	--	--	4	0	100.0

FOOD INTAKE AND CONVERSION

On day 1, the mean dry weight of the larval yolk supply was 0.027 mg (range, 0.015-0.039 mg), and on day 4, fed larvae retained 0.003 mg of yolk (range, 0-0.011 mg) while starved larvae had no yolk left.

In the quantitative feeding experiments, conducted in small, 300-ml containers, some larvae did not survive the 6-day experimental period, and some exhibited erratic swimming behavior. Only data from the surviving larvae which displayed normal behavior have been retained. Larvae did not begin feeding until day 2, although food was available to them on day 1. The number of nauplii consumed daily per larva increased as larvae grew, from less than 50 in first-feeding larvae to almost 300 in larvae 2 weeks old and older. Table 5 gives the total food consumption, growth, and conversion efficiencies of all healthy larvae which survived the feeding experiments in small containers. Larvae which displayed growth comparable to that of larvae in 10-liter containers may be expected to give the most reliable conversion efficiency values and are identified by asterisks in Table 5. One larva, fed from day 1, showed the extremely high efficiency of 73%. Table 5 suggests a trend toward decreasing conversion efficiency as larvae get older. In the experiment begun on day 7, the previously unfed larva for which data are available showed a much higher efficiency than the previously fed larva.

BODY COMPOSITION

Results of the analyses of carbon, hydrogen, nitrogen, and ash in sampled larvae are given

TABLE 5.—Food consumption, growth, and conversion efficiencies of individual larvae in small containers during 6-day feeding experiments. Asterisks identify larvae which exhibited growth comparable to that of larvae in large containers and hence probably provide the most reliable conversion efficiency figures.

Age at start of feeding experiment (days)	Previous treatment	Dry weight (mg)				Percent conversion efficiency [= (gain/total food consumed) × 100]
		Total food consumed	Larva, initial	Larva, final	Larva, gain	
1	--	[1]0.433	0.362	0.678	0.316	73.0*
1	--	[1]0.771	0.362	0.700	0.338	43.8*
4	fed	[2]1.321	0.428	1.189	0.761	57.6*
4	unfed	1.074	0.386	0.880	0.494	46.0
7	fed	1.053	0.771	0.962	0.191	18.1
7	unfed	1.026	0.409	0.826	0.417	40.6
10	fed	2.250	1.027	1.940	0.913	40.6*
10	fed	1.640	1.027	1.528	0.501	30.5*
10	unfed	0.978	0.355	0.668	0.313	32.0
13	fed	2.000	1.340	1.657	0.317	15.9
16	fed	2.365	1.517	2.002	0.485	20.5
		2.155	1.517	2.418	0.901	41.8

[1] Includes 0.027 mg of yolk.
[2] Includes 0.003 mg of yolk.

TABLE 6.—Carbon, hydrogen, nitrogen, and ash in larval samples, as percentages of total dry weight.

Age (days)	Treatment	C (%)	H (%)	N (%)	Ash (%)
1	--	45.7	7.0	10.2	5.1
4	fed	45.5	7.0	10.5	5.4
7	fed	46.2	7.1	10.5	7.9
10	fed	47.1	7.1	10.8	8.6
13	fed	46.2	7.2	11.3	8.6
16	fed	45.2	7.0	10.9	8.7
19	fed	45.0	7.0	10.7	9.2
25	fed	43.1	6.6	10.9	10.0
4	unfed	46.5	6.9	10.4	6.6
7	unfed	44.5	6.9	10.6	7.8
10	unfed	44.0	6.8	11.1	9.4
13	unfed	44.0	6.8	11.2	9.2
16	unfed	43.2	6.4	11.3	7.3
20	fed from day 1	45.0	6.7	11.3	9.9
20	fed from day 4	43.1	6.7	11.1	9.8
20	fed from day 7	44.8	6.8	11.4	9.4
20	fed from day 10	44.8	6.7	10.8	8.2
20	fed from day 13	44.6	6.7	10.9	8.8
20	fed from day 16	43.3	6.5	10.6	11.4

in Table 6. In this paper the term *level* will be used in the sense of Giese (1969) to denote the percentage of the total dry weight which a particular body component constitutes. In fed larvae, the level of ash increased from 5.1% on day 1 to 10.0% on day 25. The ash level was higher in unfed than in fed larvae, except on days 7 and 16. The nitrogen level increased with age in both fed and starved larvae, but the increase was more steady in the latter. Fed larvae fluctuated between 10.7 and 11.3% nitrogen from day 10 to day 25. Accompanying the increase in nitrogen was a decrease in the level of carbon. Among 20-day-old larvae, the level of ash was higher, nitrogen lower, and carbon the same or slightly lower in larvae whose initial feeding had been delayed for 16 days than in larvae fed earlier. Nitrogen in 20-day-old larvae decreased with time of first feeding, from day 7 to day 16. The ratio of carbon to nitrogen has been plotted in Figures 3 and 4 along with condition factors. The C/N ratio is lower in starved than in fed larvae after day 4 but shows a decreasing trend with time even in fed larvae whose condition factor is increasing (Figure 3). On day 20, larvae whose initial feeding had been delayed 10 or more days had higher C/N values than larvae fed earlier; here too, decreasing condition factors accompanied increasing C/N values. Since preservation in Formalin has been shown to affect the C, H, N, and ash levels of copepods

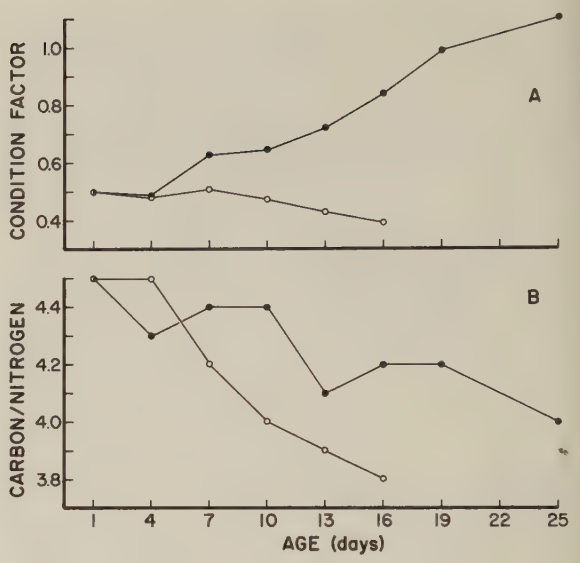

FIGURE 3.—Condition factors and carbon/nitrogen ratios of fed and unfed larvae. Condition factors were calculated as [(mean dry weight, mg)/(mean standard length, mm)] $\times 10^3$. Closed circles = fed larvae, open circles = unfed larvae.

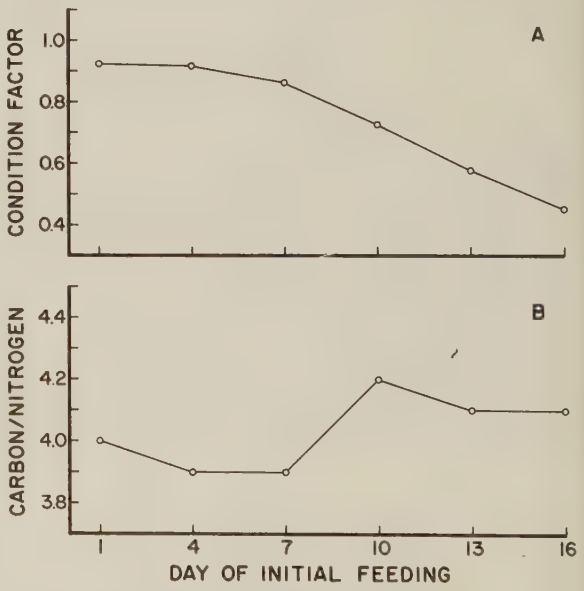

FIGURE 4.—Condition factors and carbon/nitrogen ratios of 20-day-old larvae with different times of initial feeding. Condition factors calculated as in Figure 3.

(Omori, 1970), the values given here for larvae 1 and 4 days old, which had been preserved in Formalin to allow removal of yolk by dissection, may be somewhat in error.

The level of protein in larval samples was estimated by multiplying the nitrogen level by 6.25 (White, Handler, and Smith, 1968), and fat was calculated by difference: 100 - (percent ash + percent protein) = percent fat. Nonprotein nitrogen and carbohydrate were assumed to be present in negligible amounts in this material (Lasker, 1962). Caloric content was calculated by multiplying weights of fat and protein in average larvae by 9.5 cal/mg and 5.7 cal/mg, respectively (Brody, 1945; Kleiber, 1961). Table 7 lists the resulting values. The most notable trends are an increase in protein level and decrease in fat level, in both fed and unfed larvae (Table 7). Unfed larvae had lower fat and about the same or somewhat higher protein levels than fed larvae. Among 20-day-old larvae, those for which initial feeding had been delayed tended to have higher fat and lower protein levels than those fed early (Table 7).

TABLE 7.—Protein, fat, and caloric content of larval samples. Protein and fat are given as percentages of total dry weight. Protein was calculated from the nitrogen content of samples, fat by difference, and caloric content by multiplying weights of protein and fat by standard conversion factors.

Age (days)	Treatment	Protein (%)	Fat (%)	Caloric content	
				cal/mg of dry weight	cal/mg of ash-free dry weight
1	--	63.8	31.1	6.60	6.97
4	fed	65.6	29.0	6.50	6.86
7	fed	65.6	26.5	6.25	6.79
10	fed	67.5	23.9	6.12	6.70
13	fed	70.6	20.8	6.00	6.54
16	fed	68.1	23.2	6.11	6.68
19	fed	66.9	23.9	6.09	6.70
25	fed	68.1	21.9	5.97	6.64
4	unfed	65.0	28.4	6.43	6.89
7	unfed	66.3	25.9	6.26	6.79
10	unfed	69.4	21.2	5.94	6.55
13	unfed	70.0	20.8	5.98	6.60
16	unfed	70.6	22.1	6.13	6.60
20	fed from day 1	70.6	19.5	5.89	6.54
20	fed from day 4	69.4	20.8	5.94	6.59
20	fed from day 7	71.3	19.3	5.90	6.50
20	fed from day 10	67.5	24.3	6.16	6.71
20	fed from day 13	68.1	23.1	6.07	6.65
20	fed from day 16	66.3	22.3	5.89	6.64

Reflecting changes in proximate composition, the caloric content of larval tissue showed an early decrease and from day 10 on showed no increasing or decreasing trend, in both fed and unfed larvae (Table 7). From day 10 on, starved larvae tended to have a lower caloric content than fed larvae. The caloric contents of 20-day-old larvae showed no consistent trend with time of initial feeding (Table 7).

DISCUSSION

The developmental process requires a nutritional input to supply energy and raw materials. In larval grunion which receive no food, development does not progress beyond the stage reached when the yolk is absorbed, although the larvae survive well beyond this point. The process of ossification is halted and the upward flexion of the notochord does not take place in unfed larvae, while tissue resorption, supplying energy for metabolic processes during starvation, results in a slow decrease in larval mass. Fat seems to be utilized most during starvation. The amount of fat in an average starving larva decreases by 0.071 mg, or 0.689 cal, during 16 days of starvation, while protein decreases by only 0.043 mg, or 0.245 cal (Tables 2 and 7). The fat and protein levels of feeding larvae are not greatly different from those of starving larvae (Table 7), but in the former case the observed increase in protein level with time must be a consequence of rapid protein synthesis in the growing organism, whereas in the latter it reflects the utilization of the body's fat reserves.

When food is offered to unfed larvae, growth begins and proceeds at about the same rate as in larvae fed from day 1 (Figure 2). Weight and body composition at day 20 in larvae whose initial feeding was delayed for various periods is close to that of larvae fed for similar lengths of time from day 1 (Tables 2, 3, and 7), though fat is much more depleted in larvae fed for 4 and 7 days starting on days 16 and 13, respectively, than in larvae fed for 4 and 7 days starting on day 1. Larvae fed for a period of 16 days, from day 4 to day 20, gained more weight and had higher protein levels than larvae fed from days 1 to 16 (Tables 2, 3, and 7), suggesting that a few days' delay in initial feeding caused

an increase in conversion efficiency. A similar effect has been found in adult fish (Ivlev, 1939; Pandian, 1967). There is some indication in the results of the quantitative feeding experiments that larvae convert food more efficiently after 7 days without food than after 7 days of feeding (Table 5), but the data are too meager to justify any conclusion on this point.

Omori (1970) showed that copepods from areas poor in food tended to have lower C/N ratios than copepods from rich areas. In larval fishes, condition factors have been used in attempts to assess nutritional state (Hempel and Blaxter, 1963; Blaxter, 1965). Both measures were compared in the present study (Figures 3 and 4). Although starved larvae had lower C/N ratios than fed larvae after day 4, due presumably to catabolism of fat, the C/N ratios of growing, fed larvae decreased with age as a consequence of the rapid elaboration of protein, while their condition factors increased. Reflecting this same tendency, larvae 20 days old had higher condition factors but lower C/N ratios, the longer they had been feeding. Condition factor seems to be somewhat more consistent and reliable as an index of the nutritional state of larval grunion than C/N ratio.

In sum, larval grunion appear to be extremely resistant to food deprivation. Under laboratory conditions it takes 3 weeks for all larvae to die of starvation at 18° C (Figure 1). No matter how long initial feeding is delayed, over 40% of the larvae alive when food is offered will survive, and all larvae which survive 16 days without food can commence feeding at this time and survive (Table 1). Since grunion larvae hatch from eggs deposited in the beaches of southern California and northern Baja California and must inhabit inshore waters almost exclusively, and since the abundance of microplankton is extremely high in inshore as compared with offshore waters in this region (Beers and Stewart, 1967), it seems unlikely that these larvae ever experience high rates of mortality due to starvation. Major sources of mortality among grunion larvae must be sought, rather, in predation and physical damage from waves. Tidal variations may result in different incubation periods in grunion eggs from different spawnings (Walker, 1952), but the effect of this on larval viability has yet to be determined.

These findings differ from results for clupeoid larvae. In the northern anchovy a delay in initial feeding of 2.5 days after yolk absorption resulted in nearly complete mortality, even though many larvae were alive when food was administered (Lasker et al., 1970). This "point of irreversible starvation" appears not to exist for larval grunion, as starvation can in fact be reversed at any point along the survival curve of starved larvae (Figure 1).

Larvae of the herring (*Clupea harengus*) show a decrease in the percentage of larvae which commence feeding as the period of food deprivation is lengthened, and the point at which the percentage feeding is half that at the start of the experiment has been termed the "point of no return" (Blaxter and Hempel, 1963; Blaxter, 1965). Again, the grunion larvae show a different pattern, with at least 80% of the larvae commencing feeding when food is offered after periods of starvation ranging from 7 to 16 days (Table 4). Some larvae which did commence feeding after 7, 10, and 13 days without food were nevertheless unable to survive and died after gorging themselves with *Artemia* nauplii. The interesting fact that all of the larvae alive after 16 days of starvation commenced feeding and survived, while the percentage feeding was lower in larvae starved for shorter periods of time, may be explained as a result of mortality among the weakest larvae, so that by day 16 only the most hardy individuals were still alive.

Thus, certain types of larvae would be more likely than others to show a "critical period" pattern of mortality at sea under conditions of low food availability. If northern anchovy larvae were not to encounter food within 2.5 days after yolk absorption, there would ensue a catastrophic mortality concentrated in time (Lasker et al., 1970). In contrast, grunion larvae, which hatch in a more well-developed and robust state, would exhibit mortality extending over a number of days if deprived of food and hence would not show a "critical period" in the classical sense of Hjort. Obviously a sudden increase

in mortality at sea could come about after yolk absorption, or at any other time, owing to factors other than the availability of food. Hjort was, of course, not referring to larvae of the atherinid type when he enunciated his "critical period" hypothesis, but with the large volume of published material now available concerning the larvae of a few commercially important species, it would be easy to lose sight of the great diversity of larval forms and to apply ideas which may have validity in some groups to groups in which they have no place.

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THE RELATIVE SAMPLING PERFORMANCE OF 6- AND 10-FOOT ISAACS-KIDD MIDWATER TRAWLS

WILLIAM A. FRIEDL[1]

ABSTRACT

The relative abilities of 6- and 10-ft Isaacs-Kidd midwater trawls (IKMT) to sample macroplankton and fishes were assessed from comparable hauls taken with graded mesh nets during January and February 1967, in central Puget Sound. The plankton catch, mostly individuals 2 to 2.5 cm long, was dominated by the mysid, *Neomysis kadiakensis*. To quantify zooplankton data from the larger trawl, its cross-sectional area effective in filtering macroplankton was estimated for each month. The mean effective cross-sectional area of the 10-ft IKMT is 1.75 m^2. This implies a significant funneling of macroplankton by the forward section of the trawl.

The fishes taken were dominated numerically by Pacific herring, *Clupea harengus pallasi;* bay gobies, *Lepidogobius lepidus;* and plainfin midshipmen, *Porichthys notatus*. Herring was not taken by the 6-ft trawl; there was little apparent difference in the ability of the two trawls to capture midshipmen and gobies. Overall, the 10-ft IKMT caught more fish, more active fish, and larger fish than the 6-ft trawl. Though the 6-ft IKMT is probably adequate for studies with an emphasis on macroplankton, use of the 10-ft IKMT to sample fishes in inshore waters is preferable.

Interpretation of net haul data depends upon the capabilities and limitations of the sampling gear employed. With the plethora of equipment presently available for sampling the larger plankton and smaller nekton, comparative information on the relative sampling abilities of different gear is needed to equate results obtained with different nets.

This report deals with the relative sampling abilities of two sizes of Isaacs-Kidd midwater trawl (IKMT), a type of net widely employed in marine and freshwater investigations. The results apply to IKMT in general; the assessment elucidates the degree to which data obtained with different trawls are comparable.

METHODS AND MATERIALS

Samples were taken from the University of Washington 65-ft research vessel *Hoh* at night along a N-S track in Port Orchard, a narrow channel west of Bainbridge Island in central Puget Sound with a maximum depth of slightly over 40 m. Comparable IKMT hauls were made on two cruises in January and February 1967. Length frequency data are from five cruises made each month from November 1966 through March 1967.

Two sizes of IKMT with graded mesh nets were compared. The mouth area of the 10-ft IKMT (Figure 1) is 7.68 m^2 and that of the 6-ft IKMT (Aron, 1959) is 2.94 m^2. Mesh sizes for the various sections of the trawls and other

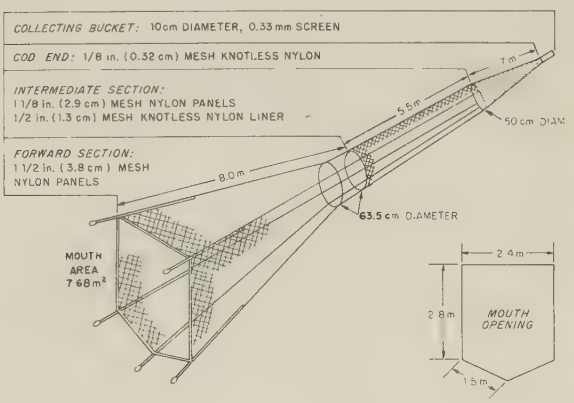

FIGURE 1.—Dimensions and construction details of the 10-ft Isaacs-Kidd midwater trawl used in this study (after Cooney, 1967).

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TABLE 1.—Dimensions and material specifications of the Isaacs-Kidd midwater trawls compared in this study.

Item	6-ft IKMT	10-ft IKMT
Mesh size		
Forward section	7.6 cm	3.8 cm
Intermediate section	1.3 cm	1.3 cm
Cod end	3.2 mm	3.2 mm
Cross-section area		
Mouth	2.94 m^2	7.68 m^2
Liner	1.26 m^2	0.32 m^2
Cod end	0.20 m^2	0.20 m^2
Filtering area		
Forward section	14.85 m^2	51.96 m^2
Intermediate section	7.02 m^2	9.81 m^2
Cod end	1.61 m^2	1.61 m^2

dimensional data are presented in Table 1. During each haul, speeds were measured at the surface with a Tsurami-Seiki-Koshakusho Co. flowmeter[2] while trawls were at depth. The same engine speed was used for all hauls. Generally, trawls were at depth 10 min and in the water less than 15 min total. In January, several 10-ft IKMT hauls were at depth 15 min. Net depth was monitored on deck from signals transmitted through the towing cable by a pressure-activated sensing unit (designed and built by the Department of Oceanography, University of Washington) mounted above the trawl. A Marine Advisers bathykymograph attached to the trawl bridle was read after each haul to check sampling depth.

The sampling distance was calculated from the speed and duration of each haul. This distance was multiplied by the appropriate trawl mouth area (Table 1) to determine the maximum volume of water filtered during each haul; the volumes so determined were used to calculate the monthly fish concentrations. For determinations of zooplankton concentrations, however, use of filtered volumes based on trawl mouth areas would result in concentrations inordinately low (Banse and Semon, 1963). Instead, volumes filtered should be based on the trawl cross-sectional area effective in sampling zooplankton of the size considered. The effective cross-sectional area, as used here, may be defined as that area which yields the correct zooplankton concentration, as measured independently, when divided into the zooplankton catch per unit distance of tow. Thus, if the length of tow is known, the number of animals caught per unit distance towed can be converted to concentration if the effective cross-sectional area is known. Banse and Semon (1963) compared euphausiid catches from a quantitative high speed catcher with those of the 6-ft IKMT and determined the effective cross-sectional area of the trawl to be not significantly different from the area of the opening of the middle (1.3-cm mesh liner) section of the trawl, namely 1.26 m^2 (Table 1). This effective area, multiplied by the sampling distance for 6-ft IKMT hauls, produced the effective volume of water filtered by the smaller trawl. Macroplankton concentrations were calculated for January and February from total 6-ft IKMT catch and total effective volumes filtered each month. Total 10-ft IKMT macroplankton catch was divided by the total sampling distance to determine the monthly macroplankton catch per kilometer by the larger trawl.

[2] The use of trade names is merely to facilitate descriptions; no endorsement is implied.

RESULTS AND DISCUSSION

Macroplankton samples from each trawl were compared to determine the effective cross-sectional area of the 10-ft IKMT. The mysid, *Neomysis kadiakensis*, represented 80% of the total catch; the mysid (*Acanthomysis macropsis*), the euphausiids (*Euphausia pacifica* and *Thysanoessa raschii*), and decapods of the genus *Crago* made up most of the rest. Most of the individuals were between 2 and 2.5 cm long. Plankton concentrations were considerably reduced in February (Table 2) and separate estimates of the 10-ft IKMT effective cross-sectional area were made for each month. Using the method of Banse and Semon (1963), division of the 10-ft IKMT catch per kilometer by the catch per 1000 m^3 filtered by the effective cross-sectional area of the smaller trawl yielded an estimate of the 10-ft IKMT effective cross-sectional area for each month (Table 2). The mean effective area, weighted according to the number of 10-ft IKMT hauls made each month, is 1.75 m^2 (Table 2). This is considerably larger than the area of the intermediate section open-

TABLE 2.—Summary of macroplankton catch data used to compute the 10-ft IKMT cross-sectional area effective in sampling macroplankton. Separate estimates were made for each month. The mean 10-ft IKMT effective area is computed from the monthly estimates and is weighed according to the number of hauls made with the larger trawl each month (see text).

Month	6-ft IKMT				10-ft IKMT				
	Hauls (no.)	Catch (no.)	Volume (10^3 m^3)	Concentration (catch/10^3 m^3)	Hauls (no.)	Catch (no.)	Distance (km)	Catch/km	Effective area (m^2)
Jan.	8	588	16.16	36.38	6	774	12.95	59.77	1.643
Feb.	8	128	17.41	7.35	12	221	16.62	13.30	1.810

Weighted mean effective area: $\frac{(1.643 \times 6) + (1.810 \times 12)}{18} = 1.754$ m^2

ing (0.32 m^2; Table 1) and indicative of a significant funneling by the forward section of the trawl.

The effective macroplankton sampling area of the 6-ft IKMT corresponds to the area of the opening of the intermediate section (1.3-cm mesh liner); effects of funneling by the forward section (7.6-cm mesh) of the trawl are not obvious (Banse and Semon, 1963). My results indicate that the forward section of the 10-ft IKMT, with 3.8-cm mesh, is relatively more important a factor in the ability of the net to sample macroplankton than the corresponding section of the smaller trawl. Given forward sections of the same mesh for both trawls, however, the effective macroplankton sampling area of the 6-ft IKMT would probably equal or exceed that of the 10-ft IKMT, for organisms of the size considered here. The ratio of the effective cross-sectional area to total trawl mouth area is 0.43 for the 6-ft IKMT and 0.23 for the 10-ft trawl. Thus, a larger percentage of the water entering the mouth of the smaller trawl is filtered for macroplankton. For this reason, and because the 6-ft IKMT is generally easier to handle and deploy, the smaller trawl would be preferred for studies with primary emphasis on macroplankton or small fishes.

The relative ability to each trawl to sample fishes was also assessed. Total catch figures are presented in Table 3. Numerically, Pacific herring, *Clupea harengus pallasi;* bay gobies, *Lepidogobius lepidus;* and plainfin midshipmen, *Porichthys notatus,* dominated the overall catch. Herring and gobies were common in hauls above 23 m while midshipmen were most abundant in deeper tows. A few shiner perch, *Cymatogaster aggregata,* Pacific cod, *Gadus macrocephalus,* spiny dogfish, *Squalus acanthias,* and miscellaneous flatfishes were also taken and are included in the category "Others" in Table 3. Though the total catch of the 10-ft IKMT exceeds that of the 6-ft IKMT for each category of fishes, the data are not directly comparable because the larger trawl filtered more water in each stratum. To equate catch data between trawls on the basis of equal volumes filtered, the 10-ft IKMT catch for each category of fish was multiplied by the ratio of 6-ft to 10-ft IKMT volumes filtered for each stratum (0.31 for hauls above 23 m; 0.47 for deeper hauls. Exact volumes given in Table 3). The product, expressed

TABLE 3.—Summary of fish catch data, by trawls, from hauls made in January and February 1967. Total catch figures are in whole numbers and concentrations of fishes (Individuals/1000 m^3 of water filtered) are in parentheses. Estimated 10-ft IKMT catch for volumes filtered equal to those of the 6-ft IKMT within each stratum is entered as "Equivalent 10-ft catch" and is directly comparable to the 6-ft IKMT catch data (see text).

Trawl	10 to 22 m						23 to 31 m					
	Hauls (no.)	Volume (10^3 m^3)	Herring	Bay gobies	Midship-men	Others	Hauls (no.)	Volume (10^3 m^3)	Herring	Bay gobies	Midship-men	Others
10-ft	14		27	24	4	8	4		1	10	48	11
		171.4	(.16)	(.14)	(.02)	(.05)		53.7	(.02)	(.19)	(.89)	(.20)
6-ft	11		0	2	0	1	5		0	2	22	1
		53.4	--	(.04)	--	(.02)		25.1	--	(.08)	(.88)	(.04)
Equivalent 10-ft catch			8	8	1	3			1	5	22	5

to the nearest whole number, is entered as "Equivalent 10-ft catch" in Table 3 and is directly comparable to the catch figures for the 6-ft IKMT in the row above it. Figures for the overall fish concentrations, from data on total catch and total volume filtered, are also given in Table 3.

When the catch data are compared on an equal volume filtered basis, the superior sampling ability of the 10-ft IKMT is evident. With the exception of *Porichthys* in the lower stratum, the 10-ft IKMT caught more fish of each category than did the 6-ft trawl and the resulting overall concentrations estimated by the 10-ft IKMT are likewise higher (Table 3). Pacific herring, a major component of the mid-depth sonic scattering layer in Port Orchard (Cooney, 1967; Friedl, 1970), were not taken by the 6-ft IKMT and apparently were capable of actively avoiding the smaller trawl. The less active, *Porichthys*, however, was sampled equally by the trawls in the lower stratum. Though the catch and concentrations of *Lepidogobius* appear much lower for the 6-ft IKMT in Table 3, the discrepancy may reflect gear selection and loss through the 7.6-cm mesh of the 6-ft IKMT forward section more than active avoidance of the trawl by the fish. The *Lepidogobius* captured were small, 35 to 50 mm SL, and probably were filtered only by the 1.3-cm mesh liner of the 6-ft IKMT intermediate section. Assuming all gobies taken by the 6-ft IKMT were filtered by the intermediate section only, the concentrations above and below 23 m would be 0.12 and 0.11 fish per 1000 m^3, respectively, and would more nearly approximate those of the larger trawl (Table 3). Thus, as with the macroplankton, the finer mesh of the forward section of the 10-ft IKMT enhanced the ability of the trawl to sample small organisms.

The 10-ft IKMT caught more fish and sampled active fish better than the 6-ft IKMT despite the fact it was generally towed at lower speeds (Table 4). The towing speed of the smaller trawl, though only slightly greater than that of the 10-ft IKMT, could have increased pressure waves and vibrations associated with the trawl and evoked greater avoidance responses

TABLE 4.—Net speed data for 6- and 10-foot IKMT hauls in January and February 1967. Speeds measured at the surface while the trawls were at depth. Average speeds are significantly different at the 99% level when compared with Student's *t* distribution (Simpson, Roe, and Lewontin, 1960).

Trawl	Hauls (no.)	Trawling speed (m/sec)		
		Range	Average	Confidence interval (95%)
10-ft	18	1.64 to 2.13	1.84	1.78 to 1.90
6-ft	16	1.99 to 2.25	2.14	2.09 to 2.19

in active fishes such as herring (Chapman, 1964; Harrisson, 1967). At present, knowledge of the pressure and vibration characteristics of IKMT underway is lacking and further conclusions regarding the influence of such characteristics on the sampling abilities of trawls would be speculative and beyond the scope of this paper.

Comparison of the length frequencies of *Porichthys* taken in deeper tows during the entire winter period (November - March) indicates a selection for larger fish by the larger trawl, despite its slower towing speeds and finer mesh (Figure 2). Nearly 70% of the *Porichthys* taken between 20 and 35 m by the 6-ft IKMT in the winter were less than 150 mm long (SL), while half the *Porichthys* taken by the 10-ft IKMT between 25 and 35 m in the same period exceeded 180 mm SL (Figure 2).

My results indicate the 10-ft IKMT catches more fish, more active fish, and larger fish than the 6-ft IKMT used in the study; similar conclusions, with respect to the ability of small and large trawls to sample mesopelagic fishes, were reached by Harrisson (1967). Aron and Collard (1969) studied the influence of net speed on catch for a 6-ft IKMT fully lined with 1.2-cm mesh netting and found that faster tows took larger fish of certain types off the California coast. My data, for inshore fishes, indicate mouth size, towing speed, net mesh size, and, perhaps, the dynamic characteristics of trawls combined with the behavioral aspects of the organisms sampled are all interrelated in a complex way to ultimately determine the sampling ability of a given trawl.

Standardization of gear and techniques used to sample midwater organisms would provide

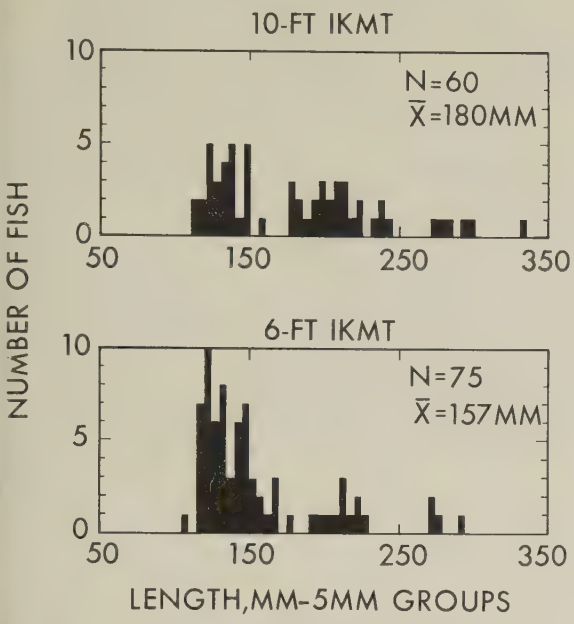

FIGURE 2.—*Porichthys notatus* length frequencies (SL) from hauls made on monthly cruises from November 1966 through March 1967. Ten-ft IKMT catch from five hauls between 25 and 35 m; volume filtered 57,400 m^3. Six-ft IKMT catch from 12 hauls between 20 and 35 m; volume filtered 58,000 m^3. Total catch (N) and mean length ($\bar{X}$) given on graphs for each trawl.

a more valid basis for comparison of different samples, but limitations of resources and equipment often determine the manner and means by which samples are taken. In this study, for instance, the strain of towing the 10-ft IKMT severely taxed the running rigging of the research vessel and prevented the use of the larger trawl on some cruises. At best, results of studies, such as this and that of Aron and Collard (1969), illuminate the limitations and capabilities of sampling gear, characteristics which must be recognized even with widely employed equipment such as the 6-ft IKMT. That trawls may sample only a limited portion of the fauna present is obvious and must be recognized. For instance, recent work in Puget Sound with large trawls indicates concentrations of herring, determined by IKMT hauls in winter sonic scattering layers, may be at least two orders of magnitude low (T. S. English, unpublished data). Thus, discussion of the biological "universe" defined by samples from a given trawl must acknowledge the limitations of the gear employed and avoid conclusions beyond the scope of the data available from the sampler.

In general, my results indicate the 10-ft IKMT to be preferable to the 6-ft IKMT for biological surveys emphasizing fishes in inshore waters, provided the vessel employed is capable of handling the large trawl on a regular basis. For surveys of macroplankton, however, the 6-ft IKMT is adequate and generally easier to deploy. The larger mouth opening and overall finer mesh of the forward section apparently enable the 10-ft IKMT to sample more fish, larger fish, and more active fish better than the 6-ft trawl. Fully lining the trawls with fine-mesh netting (UNESCO, 1968) would help simplify analysis of results by eliminating the need for estimating effective sampling cross-sectional areas when calculating concentrations of small fishes and macroplankton. Such lining would probably also increase the trawls' ability to sample smaller fishes (Backus et al., 1970), but the overall capabilities of the trawls to sample large or active forms would likely change little and utilization of the 10-ft IKMT would still be recommended.

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STUDIES ON THE USE OF CARBON DIOXIDE DISSOLVED IN REFRIGERATED BRINE FOR THE PRESERVATION OF WHOLE FISH

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ABSTRACT

Although storing fish in refrigerated seawater has many advantages over storing them in ice, the use of refrigerated seawater also has several disadvantages, one of which is the difficulty in controlling the growth of spoilage bacteria in the fish. Reported here is the effect on the growth of bacteria in rockfish and chum salmon of dissolving carbon dioxide in brine. Storing the fish in the refrigerated brine treated with carbon dioxide inhibited the growth of the bacteria, retarded the rate at which the fish decrease in quality, and increased their storage life by at least 1 week.

Refrigerated seawater as a medium for cooling, storing, and transporting fish has many advantages, which have been well documented (Idyll, Higman, and Siebenaler, 1952; Osterhaug, 1957; Cohen and Peters, 1962; Peters and Dassow, 1965; Roach et al., 1967).

This medium, however, also has disadvantages. These include the excessive uptake of water by species of low oil content, such as sole and cod, and an increase in total salt. Controlling the growth of spoilage bacteria in fish stored in refrigerated seawater also presents a problem (Roach et al., 1967). This problem results from the blood, dissolved protein, and visceral contents accumulated in the seawater during the storage of the fish. For these reasons, fish held in refrigerated seawater are not necessarily of better quality than are those held for the same period in ice. Nor can fish necessarily be held longer in refrigerated seawater than in ice before spoilage occurs.

This laboratory recently began a study of methods for increasing the effectiveness of refrigerated seawater as a medium for preserving fish. The investigation is timely because fishermen are finding it increasingly difficult to locate catches on traditional fishing grounds. This reduced abundance requires longer stays at sea, which sometimes result in the landing of fish of less than optimum quality.

Use of carbon dioxide gas dissolved in refrigerated seawater seemed promising as an inhibitor of the spoilage bacteria. Stansby and Griffiths (1935), for example, found that whole haddock and haddock fillets stored in an atmosphere of carbon doxide kept almost twice as long as did those stored in air. Castell (1953) demonstrated that carbon dioxide showed promise of being a useful preservative for salted fish held in 12% brine. Carbon dioxide has been used effectively to extend the storage life of refrigerated meat and poultry products (Wheaton, 1960) and is known to have bacterial inhibiting properties (King and Nagel, 1967). Fiskeriministeriets Forsøgslaboratorium (1968) noted that, in limited experiments on holding fish in tanks, carbon dioxide decreased the rate at which their quality was degraded. Wayne I. Tretsven (1968, personal communication) showed that the shelf life of fresh silver salmon refrigerated in a mixed atmosphere of carbon dioxide, oxygen, and nitrogen was significantly extended beyond that of fresh silver salmon refrigerated in air.

Rockfish is normally iced aboard the fishing vessel and may be held for as long as 7 to 10 days before being landed. Chum salmon is frequently held in refrigerated brine aboard cannery tenders and may be held aboard the vessel for as long as 7 days. With both methods of holding, the quality of the fish may be poor if they must be held for longer periods. This

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study is specifically concerned with the effects that holding in modified refrigerated brine containing dissolved carbon dioxide[2] has on the storage life and quality of rockfish and chum salmon.

PRINCIPLES OF THE MODIFIED REFRIGERATED BRINE SYSTEM

EFFECTS OF DISSOLVING CO_2 IN REFRIGERATED BRINE

Carbon dioxide is a relatively inert chemical compound. It is almost odorless and, in the gaseous form, is colorless. Combined with water, it forms carbonic acid, a weak acid. Depending on the conditions, only part of the CO_2 added to the water is dissolved. The undissolved CO_2 either rises to the surface of the solution and is wasted away or else becomes suspended as gas bubbles, thereby forming carbonated water. The amount of CO_2 that can be dissolved by water depends on the pressure and temperature. The higher the pressure of the CO_2 and the lower the temperature of the water (at least, down to 35° F), the greater the amount of CO_2 dissolved. (We found that lowering the temperature below 35° F did not increase solubility.)

When chilled brine is saturated with CO_2, its pH is reduced from about 7.5 or higher to about 4.0. This change in pH from the alkaline to the acid condition helps to inhibit the growth of bacteria that contribute to spoilage (Wheaton, 1960). But pH control is not the only operative factor. Dissolved CO_2 seems to inhibit the metabolic processes of spoilage organisms and, of course, temperature control is important in slowing growth rate.

Although the modified refrigerated brine technique produces positive effects with regard to the control of bacteria, the addition of CO_2 can, under certain conditions, produce undesirable side effects. These effects are manifested in the form of accelerated corrosion rates of metals exposed to seawater containing high concentrations of the dissolved CO_2.

[2] In this report, the term "modified refrigerated brine" will henceforth mean brine containing dissolved carbon dioxide (CO_2).

REFRIGERATED BRINE EQUIPMENT

The equipment we used consisted of two fiber glass-insulated 55-gal epoxy-coated drums and a brine chiller.

We cooled the drums by circulating refrigerated brine from the brine chiller through 200 ft of ¾-inch polyvinyl chloride tubing wound around the outside of the drums in series and returned to the chiller (Figure 1).

Polyethylene liners with a capacity of about 30 gal were suspended in the drums by clamps. (The purpose of the liners was to keep the fish away from the cold sides of the drum, where they tend to freeze.)

Each drum was equipped with a Moyno[3] pump (Figure 2) for recirculating chilled brine (a solution containing 3.3% sodium chloride).[4] The brine was circulated by the pumps through a fitting in the bottom of the polyethylene liner. It was then forced through fish that had been placed in the liners, whereupon it overflowed back into the drums. The brine in the drums was picked up by a suction hose and recycled through the pumps at the rate of 10 gal/min. For maximum diffusion into the brine, the CO_2 gas was fed into the suction side of the circulating pump at the rate of 0.2 ft^3/hr. The brine in the other drum was left untreated for use as a control.

STORAGE LIFE AND QUALITY OF ROCKFISH HELD IN MODIFIED REFRIGERATED BRINE

OBJECTIVE MEASUREMENTS

Both bacteriological and chemical measurements were made. All measurements reported here were made in duplicate.

[3] The use of trade names is merely to simplify descriptions; no endorsement is implied.

[4] Sodium chloride brine was used in lieu of natural seawater because clean seawater was not convenient to the laboratory. However, this technique has previously been used by Collins (1950), Davis and Clark (1944), and others and found to give good results. In comparative experiments conducted by Roach and Harrison (1954) and more recently by this laboratory (unpublished), the test results showed that fish held in refrigerated brine were of equal quality to fish held in refrigerated seawater.

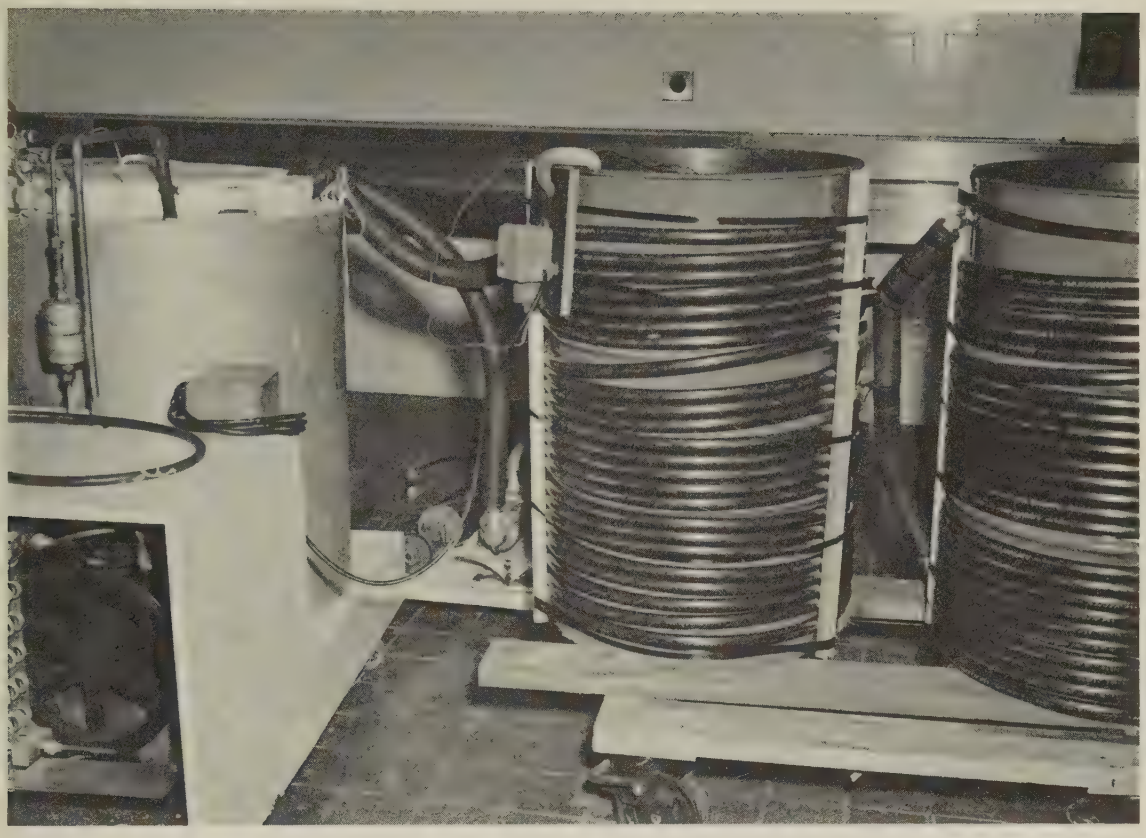

FIGURE 1.—Arrangement of the brine chiller (on the left); the pump is (on the floor) for circulating the chilled brine through the cooling coils shown wrapped around the uninsulated holding tanks.

Bacteriological Measurements

Materials and methods.—Described here are the rockfish and brine samples we used and the methods of making total plate counts.

The rockfish, *Sebastodes flavidus*, were caught in a trawl off the coast of Oregon. In the preparation of the samples, 130 lb. of the fresh, whole fish was divided into two equal lots. Each lot was placed in a polyethylene-lined drum of brine at a one-to-one ratio by weight of fish to brine. At this time, the iced fish had been out of the water 24 hr. The ratio by weight of fish-to-brine was maintained throughout the experiment by removing a known weight of brine at each sampling period.

One tank of brine was treated with CO_2 gas before the fish were loaded into it. The brine in both tanks was cooled to 31° ± 0.5° F during the experiment.

Periodically three fish and a sample of brine were removed from each of the storage drums for examination. The fish samples were used to make both the objective and subjective measurements at each sampling.

Total bacterial plate counts were made on the fish by the methods described by Pelroy and Eklund (1966). Briefly, the method was as follows: a slice of flesh was removed from near the dorsal side of each fish just posterior to the nape. Each subsequent experimental sampling was made from the same side and area of each fish tested. Forty-five grams of fish from the excised samples was homogenized aseptically

FIGURE 2.—Arrangement of the Moyno pump for recirculating chilled brine in the holding tank (on the left) and the CO_2 cylinder (in the back) and attached CO_2 flow meter (being adjusted by the worker).

with 180 ml of sterile 0.1% peptone solution at 38° F. Serial dilutions in 0.1% peptone-water were prepared for pour plates from the homogenate. Total plate counts were made by use of a TPY medium (0.5% yeast extract, 1.5% trypticase, 0.5% phytone, 0.2% glucose, 0.5% NaCl, and 1.5% agar). Counts were made on the brine by taking 1-ml samples, making appropriate serial dilutions in the 0.1% peptone-water mixture, and plating out onto the TPY medium. The plates were incubated at 22° C for 5 days.

Results and discussion.—Table 1 gives the results of the total-plate-count analyses on the brines and on the flesh of the rockfish. The data from the untreated brine show that a lag in bacterial growth occurred during the first 3 days of the storage test. After the third day, however, the population of bacteria in the brine increased rapidly.

Total plate counts made on the brine treated with CO_2 did not show a significant increase in the number of bacteria during the 17 days of storage.

Bacterial growth in the flesh of the rockfish held in the untreated brine was not inhibited during storage. On the 10th day, the fish were judged, by appearance and odor, to be inedible and unfit for testing. At this time, the total plate counts each exceeded a million organisms per gram of flesh. (A total plate count of a million organisms per gram usually indicates flesh of poor quality.)

TABLE 1.—Chemical and microbiological changes occurring in CO_2-treated refrigerated brine and in untreated refrigerated brine, and in the flesh of rockfish held in these brines.

Time in storage	Data on refrigerated brine with added CO_2							Data on refrigerated brine without CO_2				
	CO_2 conc.		pH		Salt conc.	Total bacterial plate count		pH		Salt conc.	Total bacterial plate count	
	Flesh	Brine	Flesh	Brine	Flesh	Flesh	Brine	Flesh	Brine	Flesh	Flesh	Brine
Days	*ppm*	*ppm*			*%*	*no./g*	*no./ml*			*%*	*no./g*	*no./ml*
0	119	1,000	6.7	4.0	0.2	1.2×10^4	1.3×10^4	6.7	6.8	0.2	1.2×10^4	1.3×10^4
3	562	2,332	5.8	5.3	0.5	--	1.6×10^4	6.4	6.9	0.6	7.7×10^4	1.2×10^4
8	842	1,848	6.4	6.0	1.1	1.6×10^4	--	6.5	7.3	1.0	2.4×10^8	2.8×10^8
10	--	--	--	6.0	1.3	--	--	--	--	--	Spoiled[1]	$>10^6$
14	597	1,452	6.5	6.0	--	1.4×10^4	5.3×10^5	--	--	--	--	--
17	--	--	6.4	6.1	1.8	2.0×10^4	3.8×10^4	--	--	--	--	--

[1] Samples judged by appearance and odor to be inedible and unfit for tasting.

The storage of rockfish in the refrigerated brine containing the CO_2 was terminated after 17 days. At this time, the results of the total plate counts made on the flesh of the fish showed that the microbial population had not changed significantly from the initial total plate count of 10^4 organisms per gram of flesh.

Chemical Measurements

pH.—The pH of the flesh of the rockfish and of the brines was measured by means of a Beckman combination electrode. The pH of the flesh was measured by inserting the tip of the electrode into the flesh (Patashnik, 1966).

Table 1 gives the results of the pH measurements of the fish and the brine. After 8 days of continuous recirculation, the pH of the control brine changed from a slightly acid condition (pH 6.8) to a slightly alkaline condition (pH 7.3). This change coincided with an increase in the microbial population in the control brine and was probably due to the formation of ammonia and amines from the bacterial degradation of proteins dissolved in the brine.

The initial pH of the brine treated with CO_2 shows the effect of the dissolved CO_2. The measurement was made before the fish were loaded into the brine. The subsequent increase in the pH of the brine in the presence of additional CO_2 may be attributed to the buffering by the soluble proteins in the blood and slime. After the 8th day of the experiment, the pH of the brine treated with CO_2 did not increase significantly.

Between the initial examination and that on the 3rd day, the pH of the flesh of the fish held in the brine treated with CO_2 dropped appreciably. This change was coupled with an increase in the concentration of the CO_2 in the flesh. As storage continued, the pH returned to the same level (6.4 to 6.5) as that of the flesh held in the untreated brine.

CO_2 concentration.—The concentration of CO_2 in the flesh and brine was measured by the method of Umbreit, Burris, and Stauffer (1957). The procedure was essentially as follows: A slice of fish was removed from the thickest part (dorsal side) of the fish. The sample was then carefully sectioned into horizontal cuts about ¼-inch thick and the individual cuts analyzed. A sample of the flesh or of brine was blended in Tris buffer (hydroxymethyl) aminoethane at a pH of about 9. Five grams of the mixture was added to a Warburg flask, and 0.7 ml of 0.5 M citrate buffer at pH 4.0 was added to the side arm of the flask. After the flask and its contents came to equilibrium at 38° F in a water bath, the contents of the side arm were tipped into the flask. The increase in manometric pressure was recorded at irregular intervals of time ranging up to 10 min. The amount of CO_2 evolved was calculated from a standard curve prepared by determining the changes in pressure after measured amounts of acid were tipped into known concentrations of bicarbonate.

Penetration studies carried out on whole rockfish showed that CO_2 diffused into the flesh very slowly. The maximum depth of penetration into the flesh was 0.75 inch. This depth was reached in about 8 days of storage. The highest

concentration of CO_2 in the flesh (842 ppm) was reached at this time.

The retention of CO_2 in the flesh was given consideration as a potential problem in contributing to an abnormal head-space pressure in canned salmon and to the separation of breading on breaded rockfish products. The initial indications were, however, that the retention of CO_2 will not be a problem. As was remarked earlier, CO_2 is not absorbed well at above-normal storage temperatures. CO_2 will therefore likely be dissipated from the flesh during routine cleaning, heading, and washing operations, which are done at temperatures considerably higher than those of storage. In an experiment in which red salmon were held in modified refrigerated natural seawater and commercially canned, no problems were encountered as the result of CO_2 retention. In canned products such as tuna and shrimp, the retention of CO_2 should not be a problem, because these products are exposed to relatively high preprocessing temperatures.

Additional consideration of these potential problems, however, will be given to the retention of CO_2 in future studies on modified refrigerated brine.

Salt concentration.—The concentration of sodium chloride was measured by the method described by Greig and Seagran (1965). In brief, a plastic-strip indicator containing a sensitized capillary element was placed in a filtered extract of fish and distilled water. After the reading was taken by means of the indicator, the concentration of salt in the extract was read from a standard curve supplied by the manufacturer of the device.

During the first 8 days of storage, the uptake of salt was similar in the fish held in treated brine to that in the fish held in the control brine. Concentrations of salt in the fish held in the untreated brine for longer than 8 days were not determined, because these fish spoiled at about that time. The fish held in the treated brine were analyzed for concentration of salt on the 10th and 17th days of storage. They showed somewhat more uptake of salt at each of these times.

SUBJECTIVE MEASUREMENTS

Raw Rockfish

At each sampling, a trained taste panel determined the effect of storage of the fish in two kinds of brine water. The fish were also evaluated in the round for general appearance and odor.

During the first 3 to 4 days of storage, fish held in either of the two brines were of good color, odor, and texture. By the 5th day, odors occurred in the fish held in the untreated brine. Between the 7th and 10th days, the fish were judged, by appearance and odor, to be unfit for tasting. During this time, the untreated brine had a strong odor of putrefaction and was dark brown.

The fish held in brine treated with CO_2 retained good color, odor, and texture for 17 days. The brine was almost colorless and odorless at the end of the experiment.

Cooked Rockfish

Cooked rockfish were prepared for taste-panel evaluation by the method of Miyauchi, Stoll, and Dassow (1964). The samples of cooked fish were evaluated for appearance, odor, flavor, texture, and overall quality, using a 10-point numerical scale.

Table 2 gives the sensory scores for the cooked samples. The data show that the fish in the untreated brine spoiled between the 7th and 10th days of storage. Except for an increase in saltiness, which occurred in the fish in either

TABLE 2.—Sensory evaluations on the cooked flesh of rockfish held in CO_2-treated refrigerated brine and untreated brine (control).

Time in storage	Overall sensory score[1]		Comments	
	Brine and CO_2	Brine (control)	Brine and CO_2	Brine (control)
days				
0	9.0	9.0	--	--
3	8.0	8.0	Slight salty taste	Slight salty taste
7	7.0	6.0	Unobjectionable salty taste	Off-odors
10	--	Spoiled	Unobjectionable firm texture	Foul off-odors
14	7.0	--	Firm texture	--
17	7.0	--	Objectionable salty taste; texture, color, and odor good	--

[1] A score of 10 denotes a product of highest quality; one of 5 denotes a product of borderline quality.

of the two storage environments, the fish held in the modified brine water were organoleptically acceptable and of good quality after 17 days of storage. The subsequent refrigerated shelf life of this product was not determined.

STORAGE LIFE AND QUALITY OF CHUM SALMON HELD IN MODIFIED REFRIGERATED BRINE

OBJECTIVE MEASUREMENTS

Both bacteriological and chemical measurements were made. All the reported measurements were made in duplicate.

Bacteriological Measurements

Materials and methods.—Described here are the samples of salmon and of brine and the methods of making total plate counts.

About 300 lb. of fresh seine-caught chum salmon, *Oncorhynchus keta,* were obtained in the round from Bellingham, Wash. The salmon, which weighed about 10 to 13 lb. each, were divided into two lots of equal size. Each lot was loaded at a one-to-one brine-to-product ratio by weight into a drum of circulating brine containing 3.3% NaCl (see footnote 4). The salmon had been held in ice and, at this time, were less than 24 hr out of the water.

The brine was precooled and treated as was described in the section on rockfish.

Three salmon and a sample of brine were removed periodically for examination. The fish samples were used to make both the objective and subjective measurements at each sampling.

Total bacterial plate counts were made of the bacteria on the skin of the fish. Samples of the bacteria were obtained by the swab technique of Tretsven (1963). Briefly, the procedure consisted in swabbing the skin of the fish with a sterile swab through a 2 cm^2 hole cut into the center of a sterilized metal template. The tip of the swab was broken off in such a way that it fell into 10 ml of a 0.1% peptone solution, which was then mixed. Appropriate serial dilutions were made from this mixture and were plated out on the TPY medium (see the bacteriological section described under rockfish) for the determination of total bacterial counts.

In previous experiments at this laboratory, the swab technique gave results similar to those obtained from samples of flesh. Because of this finding and because of the relative simplicity of the swab technique, we used it in this experiment. Total plate counts of the bacteria in the brine were made by the method used in the rockfish experiment.

Results and discussion.—Table 3 shows the total plate counts made on the untreated and treated brines. In the control brine, the bacterial population steadily increased during the 18-day experiment. About the 7th day of storage, the brine evidenced a slight odor of spoilage. By the 11th day, the untreated brine smelled intensely putrid. At that time, the total plate count exceeded 10^6 organisms per milliliter.

The effect of CO_2 is demonstrated by the essentially unchanged bacterial population in the treated brine during the experiment. The bacterial population increased between the 3rd and 9th days but appeared then to stabilize. During the experiment, the brine remained odorless and,

TABLE 3.—Chemical and microbiological changes occurring in CO_2-treated refrigerated brine and in untreated refrigerated brine, and on the flesh of salmon held in these brines.

Time in storage	Data on refrigerated brine with added CO_2				Data on refrigerated brine without CO_2			
	pH of brine	Salt conc.	Total bacterial plate count		pH of brine	Salt conc.	Total bacterial plate count	
			Skin	Brine			Skin	Brine
days		%	*no./cm*	*no./ml*		%	*no./cm*	*no./ml*
0	4.0	0.3	1.1×10^5	1.3×10^4	7.1	0.3	1.1×10^5	1.3×10^4
3	5.5	0.6	7.7×10^4	9.3×10^4	6.8	0.6	--	8.1×10^4
9	--	1.3	1.9×10^5	1.4×10^5	--	1.2	2.4×10^6	2.6×10^6
11	5.5	--	3.2×10^4	2.1×10^5	6.8	--	1.0×10^6	5.0×10^6
18	5.5	1.3	3.8×10^4	2.0×10^4	6.8	1.4	3.3×10^6	3.5×10^7

except for a small amount of suspended protein, remained clear and colorless.

The number of bacteria on the skin of the salmon held in the untreated brine increased more than 20 fold. After the salmon had been in storage for 9 days, the number of bacteria on the skin increased from its original value of 1.1×10^5 to $2.4 \times 10^6/cm^2$. At 11 days of storage, the salmon were judged, on the basis of odor, to be spoiled.

Swab tests on the skin of the salmon held in the brine treated with CO_2 showed that essentially no growth of bacteria occurred during the 17 days of storage.

Chemical Measurements

pH.—The pH of the brine was measured as was described earlier. The pH of the flesh was not measured. Table 3 shows the pH values for the brines.

Except for the initial value of 7.1, the untreated brine had a pH of 6.8 throughout the experiment. As yet, we do not know if the difference in pH of the brines used for holding rockfish and salmon is related to a difference in the spoilage patterns of the two species.

The pH of the brine treated with CO_2 remained in a stable acid condition throughout the experiment.

CO_2 concentration.—No analyses were made for CO_2 in the flesh or in the brine. However CO_2 was continuously metered into the experimental brine at the same rate as that in the experiment with rockfish.

Salt concentration.—For greater accuracy than is possible with the simple rapid method of analysis described earlier, the concentration of NaCl in the flesh was measured by the Volhard method (Horwitz, 1960). The sample analyzed was taken from both fillets of a single fish. The fillets were mechanically comminuted and thoroughly mixed before the sample was taken, and the analyses were made in duplicate.

As was true with the rockfish, treating the brine with CO_2 had no effect on the rate of salt uptake. Salmon held in both brines showed progressive and similar increases in concentration of salt to a maximum of 1.3% to 1.4% in the flesh at 9 days.

SUBJECTIVE MEASUREMENTS

Raw Salmon

At each sampling, the salmon were examined in the same manner as had been the rockfish.

At the beginning of the experiment, the untreated salmon had a bright appearance and a thick covering of colorless slime. After 4 to 5 days, however, they had lost their natural brightness. They remained slimy, but the slime had begun to turn yellow. By the 11th day, the salmon looked blanched and smelled spoiled. At this time, the brine was dark brown and had an intense odor of spoilage.

The salmon held in the brine treated with CO_2 retained most of their natural color during the experiment. By the end of the first week of storage, however, only a trace of slime remained on their skins. On the 18th day, when the experiment was terminated, the salmon still had a good appearance and were free of off odors. The brine was almost colorless and almost odorless.

Cooked Salmon

The taste-test scores (Table 4) show that the samples from both storage environments were equally acceptable through the first 7 days of storage.

TABLE 4.—Sensory evaluations on the flesh of chum salmon held in CO_2-treated refrigerated brine and in untreated refrigerated brine.

Time in storage	Overall sensory score[1]		Comments	
	Brine and CO_2	Brine (control)	Brine and CO_2	Brine (control)
days				
0	9.0	9.0	--	--
3	8.0	8.0	--	Slight salty taste
7	8.0	8.0	--	--
11	7.0	Spoiled	Unobjectionable salty taste; firm texture	Odor of uncooked flesh putrid
18	6.0		Color, odor, and texture good; objectionable salty taste	--

[1] A score of 10 denotes a product of highest quality; one of 5 denotes a product of borderline quality.

By the 11th day, however, the taste panel rated those held in the untreated brine as being unacceptable.

In contrast, the salmon held 18 days in the treated brine were acceptable. The panel judged that these salmon had good texture and color but that they had only fair flavor. The deterioration in the flavor may have been due in part to the presence of absorbed salt (a salt concentration of about 1.0% is generally considered to be optimum) but was due mostly to chemical changes that occurred in the flesh during storage.

SUMMARY AND CONCLUSIONS

The purpose of the work reported here was to determine the effect that holding rockfish or chum salmon in refrigerated brine treated with CO_2 would have on their storage life and quality.

Storing rockfish in brine treated with CO_2 increased their storage life by at least 1 week. The CO_2 inhibited bacterial growth and retarded the rate at which the rockfish decreased in quality.

Storing chum salmon in brine treated with CO_2 gave similar results.

This study indicates that the addition of CO_2 to refrigerated brine considerably improves the preservation properties of this medium with respect to bacterial spoilage. The absorption, however, of water, uptake of salt, loss of soluble protein, and the as-yet-undetermined subsequent refrigerated shelf life of the landed product are problems that remain to be solved. At this time, we therefore cannot recommend that rockfish and chum salmon be held in modified refrigerated brine beyond presently accepted storage periods—that is, 8 to 10 days for either species.

Although we do not at present recommend extending the holding times, the reader may wish to keep in mind that the quality of a landed product held in refrigerated brine is significantly improved by the addition of CO_2.

Future modified brine studies will be directed at solving the above mentioned problems and the problems concerned with accelerated corrosion.

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DDT RESIDUES IN SEAWATER AND PARTICULATE MATTER IN THE CALIFORNIA CURRENT SYSTEM

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ABSTRACT

Continuous samples of seawater and organic particulate material collected along linear transects in the California current system were analyzed for DDT residues. DDT residue concentrations in whole seawater, as determined by continuous-flow, liquid-liquid extraction, ranged from 2.3×10^{-12} g/ml off Oregon and Washington, to 5.6×10^{-12} g/ml off southern California. Geographical patterns in these concentration values are discussed in relation to mechanisms of land-sea DDT residue transfer. DDT residue concentrations in particulate material collected by continuous-flow centrifugation and filtration of the centrifugal pellet onto GFC-glass-fiber filters, ranged from 1.2 to 5.7×10^{-6} g/g carbon (with one exception). These values were related to the density of the standing crops. DDT residues in this particulate fraction accounted for less than 10% of the DDT residues in the whole seawater samples. Residues which are fixed to particles of less than 1-2 μ in diameter may account for the balance of the DDT residues in the whole water samples. Experimental results are described which implicate adsorption as the uptake mechanism for algal cells; these experiments also support the idea that <1-2 μ diameter particles carry most of the DDT residues in whole seawater.

DDT and its metabolites have dispersed into the ocean and are found in high concentrations in the predators of oceanic food chains. Theoretical considerations predict a net transfer of extant DDT residues to the oceans, via atmospheric and river currents (Smith, 1970). In view of the well-known chemical stability of the principal constituents of the DDT complex, *p,p'*-DDT, DDD, and especially DDE, it is not surprising that levels of DDT residues in marine plankton samples have risen during the past decade (Cox, 1970a). No published data are available, however, on concentrations of DDT residues in seawater and in oceanic particulate matter. Chlorinated pesticides have been found in concentrations up to 13×10^{-9} g/ml in surface slicks in Biscayne Bay, Fla., and at concentrations of about $^{-12}$ g/ml in the surrounding waters (Seba and Corcoran, 1969). Measurements of DDT concentration in the open ocean are needed to construct a systematic account of DDT residue transport to the pelagic environment of the ocean, and to estimate the ultimate transport of DDT residues to the sediments.

METHODS AND MATERIALS

Samples of water and particulate material were collected during cruises of the RV *Proteus* in May 1970 from Monterey Bay, Calif., to San Diego, Calif., passing outside the islands off the southern California coast and returning closer to shore through the Santa Barbara Channel. A second cruise was made in September 1970 from Vancouver, British Columbia, to just off the mouth of San Francisco Bay, Calif. Figures 1 and 2 show the cruise tracks and the station enumeration for these cruises.

Sampling was continuous and was done while the ship was underway. Water was obtained from the shipboard seawater system (PVC and Teflon) which pumped water from about 1-2 m below the surface. The stream was first filtered through a 0.176-mm mesh net to remove larger zooplankton from the sampled water. The stream was then split; part of the water was directed into a peristaltic pump which metered the flow of particle-bearing water into a continuous-flow, internal recycle and recovery, liquid-liquid extracter of the type described by Kahn and Wayman (1964). Flow rates through the liquid-liquid extracter averaged 480 ml/hr.

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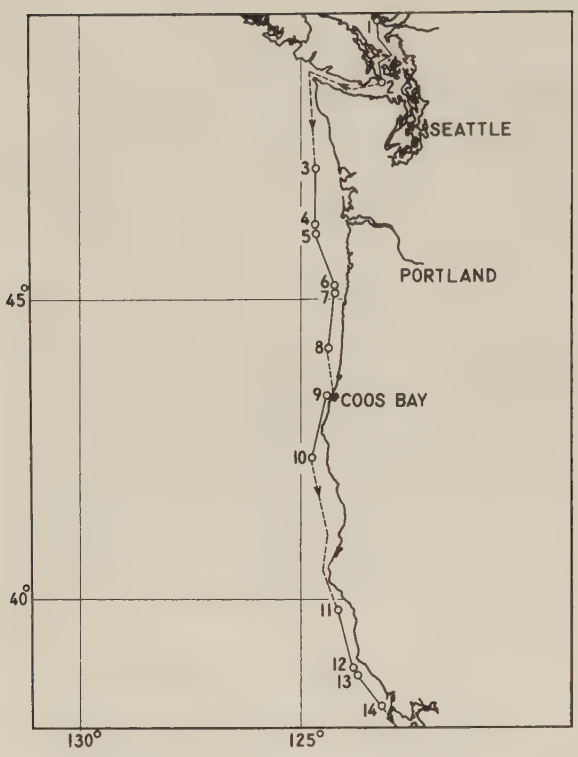

FIGURE 1.—Chart of the transects from Vancouver, British Columbia, to San Francisco Bay, Calif. See Tables 1 and 3 for station data.

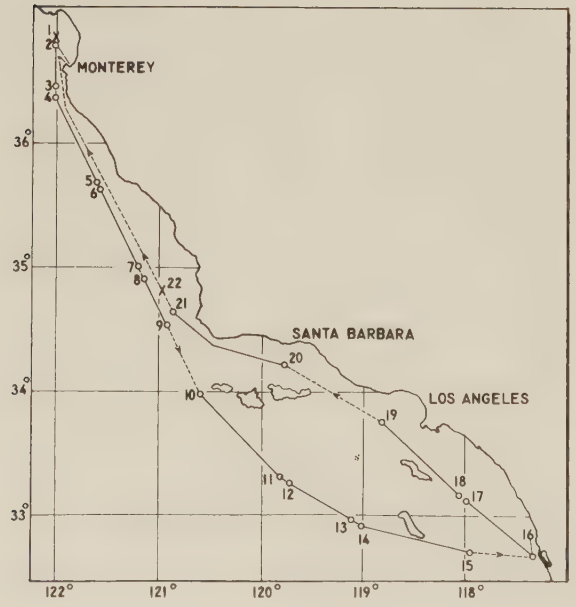

FIGURE 2.—Chart of the transects from Monterey Bay, Calif., to the southern California area. See Tables 2 and 4 for station data.

Since only one extracter was used for water extraction, the possibility existed for incomplete recovery of the DDT residues in the water passing through the device. Repeated tests of the extraction efficiency using a large carboy of oceanic seawater labelled with low levels of ^{14}C-DDT (ca. 5×10^{-12} g/ml) gave an extraction efficiency of 83% ($\pm$5%) at the flow rate settings which were used with apparatus at sea. A Variac setting of 70 was used, which produced an internal recycle rate of 900 ml/hr. The magnetic stirrer rate, which affects the degree of fracture of the solvent droplets, was kept constant.

At the end of a particular run, the contents of the centrifuge tubes were transferred to combusted 4.25 cm Whatman GFC filter papers[2] and stored in glass petri dishes at —15° C until analysis. The samples from one tube were analyzed for particulate carbon by the wet combustion method of Strickland and Parsons (1968). The samples from the other tube were analyzed for DDT residues according to previously described methods (Cox, 1970a).

At the end of a water extraction run, the flask containing about 100 ml of hexane, the water extract, was removed and stored until processing. The extract was condensed to 100 μliters after dehydration by passage through an Na_2SO_4 column. The Na_2SO_4 was specially rinsed with solvent and combusted at 350° C to remove interfering impurities normally present in the reagent salt (Lamar, Goerlitz, and Law, 1966). The condensed extract was spotted on an alumina chromatoplate, which was developed in 5% benzene in hexane, so that the solvent front moved 10 cm from the origin. Centimeter wide zones were stripped from the chromatoplates which corresponded to zones expected to contain *p,p'*-DDT, DDD, and DDE according to spots on parallel chromatograms with pure standards. ^{14}C-DDT and ^{14}C-DDE were also used to determine R_f zones. These zones

[2] The use of trade names is merely to facilitate descriptions; no endorsement is implied.

were eluted with a small amount of 20% benzene in hexane into test tubes. The eluates were analyzed individually by the same gas chromatographic techniques used for the particulate samples.

All glassware was combusted at 350° C overnight to remove interfering contaminants. All solvents were nannograde or pesticide quality. A hexane blank was run through the same procedure to detect systematic errors from any of the steps after the initial extraction. No correction was found to be necessary.

RESULTS AND DISCUSSION

WHOLE SEAWATER EXTRACTS

Comparisons of DDT residue concentrations in the particulate samples obtained by the centrifugation/filtration method described above (hereafter referred to as the particulate material) are meaningless when they purport to describe geographical differences since these concentrations change according to the density of the standing crop of the particulate material (Cox, 1970a). Comparisons of the concentrations of DDT residues in whole seawater (Tables 1 and 2) reveal some significant geographical differences. Water in the southern California region appears to have a higher DDT residue concentration. Water off Oregon and Washington has lower concentrations, and there is no evidence of high DDT residue levels adjacent to the mouth of the Columbia River. The relative uniformity of the DDT residue concentrations for this northern cruise (Table 1) suggests a diffuse source of the residues, possibly from atmospheric fallout. Direct measurements of the DDT content of dust in the atmosphere over the Atlantic Ocean (Risebrough, Hugget, Griffin, and Goldberg, 1968) and measurements of DDT residues in rainwater (Tarrant and Tatton, 1968; Yates, Holswade, and Higer, 1970) implicate aerial transport as an important mechanism of land-sea DDT residue transfer. Published calculations based on annual rainfall statistics and probable DDT residue concentrations in rainwater predict the concentration of DDT residues in the surface mixed layer of the oceans to be 5×10^{-12} g/ml (Smith, 1970). This estimate is within a factor range of 0.5 to 1.1 of the results presented in Tables 1 and 2.

Atmospheric fallout may be important in areas remote from river systems draining agricultural areas or in areas remote from waste dumping of highly populated areas. Sewage outfalls near large centers of population, such as the southern California area, contribute a large share of the DDT residue input to the ocean. When the outfall is below the pycnocline, the DDT residues in the effluent may settle with the particles comprising the solid component of the sewage and thus enter the benthic environment. This may account for the high DDT levels found in the livers of bottom dwelling fish in the southern California region, as compared to pelagic species (figures released by the California Department of Fish and Game in 1970). Sedimentation of organic particulate material from the surface layers represent an additional input to the benthos.

Input of DDT residues to the mixed layer is represented by the following sources: (1) sewage input by vertical transport of material from below the pycnocline or by direct input from shallower outfalls, (2) input from terrestrial runoff water which bears fallout particles, and (3) direct input from fallout of particles over the water. The relative importance of these

TABLE 1.—DDT residue concentrations in seawater obtained by liquid-liquid whole water extracts from transects shown in Figure 1.

Stations	Total volume extracted (liters)	DDT concentrations in water-parts per 10^{12}
1-2	2.6	2.3
3-8	4.1	2.7
9-10	2.8	2.3
11-14	4.3	2.3

TABLE 2.—DDT residue concentrations in seawater obtained by liquid-liquid whole water extracts from transects shown in Figure 2.

Stations	Total volume extracted (liters)	DDT concentrations in water-parts per 10^{12}
4-7	2.8	4.1
10-13	4.0	3.0
14-15	1.6	5.6
16-19	3.3	3.4

sources is not known, but it is quite likely that sources (1) and (2) account for the higher DDT residue concentration in the whole seawater samples taken off southern California.

PARTICULATE MATERIAL

Results of the analyses of the particulate material are shown in Tables 3 and 4. Transect 10-11 (Table 4) yielded an abnormally high value when compared to the other values for particulate material. During transect 10-11, visual observations were made of oil globules at the sea surface. The abnormally high value may have been caused by inclusion of a small globule of this material in the particulate material for transect 10-11, after entrainment in the seawater system of the vessel. This value has been deleted from further data presentations.

TABLE 3.—DDT residue concentrations in organic particulate material collected by continuous-flow centrifugation and collection of the centrifugal pellet on GFC-glass-fiber filter papers. Transects shown in Figure 1.

Transect stations	Total volume filtered (liters)	Wt. of carbon in centrifugal pellet (g × 10—6)	DDT concentration μg DDT residues/g carbon (ppm)
1-2	48	4,500	1.4
3-4	48	2,980	2.2
5-6	29	2,550	1.8
7-8	17	490	5.7
9-10	39	2,680	2.1
11-12	24	1,780	2.3
13-14	28	600	8.3

TABLE 4.—DDT residue concentrations in organic particulate material collected by continuous-flow centrifugation and collection of the centrifugal pellet on GFC-glass-fiber filter papers. Transects shown in Figure 2.

Transect stations	Total volume filtered (liters)	Wt. of carbon in centrifugal pellet (g × 10—6)	DDT concentration μg DDT residues/g carbon (ppm)
1	(1)	1725	27.0
2-3	44	4700	1.6
4-5	36	2130	2.4
6-7	37	2750	2.4
8-9	23	2140	2.5
10-11	36	3320	16.0
12-13	19	2330	1.5
14-15	32	2690	1.4
16-17	50	3280	1.6
18-19	60	3030	1.2
20-21	42	4010	1.3
22	(1)	3770	1.2

[1] These samples were obtained by using a net; see text for details.

On the May cruise to southern California (Figure 2), two phytoplankton ¼-m net tows (35-μ effective aperture) were taken at stations 1 and 22, and analyzed along with the particulate material samples. These tows consisted of 10 successive vertical hauls from 15 m to the surface at station 1 and one oblique haul from 10 m to the surface at station 22. The station 1 value is in approximate agreement with earlier published DDT residue concentrations for net phytoplankton samples (27 ppm per unit of carbon converts to 0.27 ppm wet weight; compare to values given by Cox, 1970a). This value is considerably higher than the values listed in Tables 3 and 4 for particulate material. At station 22, the ship was stopped for an investigation of a dense phytoplankton bloom, which consisted principally of *Rhizosolenia* spp. No measurements of chlorophyll were made, but the water was visibly discolored due to the high concentration of algal cells in parallel streaks at the surface. The concentration of DDT residues in net-tow material from this bloom was considerably lower than in the sample taken at station 1 (0.012 ppm wet weight compared to 0.27 ppm). This may be explained by the fact that the standing crop density was much higher at station 22 than at station 1.

The generally lower values in the particulate material compared to net-tow material (except in the case of station 22 as discussed above) could result from at least three causes: (1) loss of materials by cells bursting during the centrifugation (filtration as a cause of bursting of cells is well known, but cannot account for a difference in this case since the net-tow samples [Cox, 1970a and this report] were vacuum filtered through GFC papers as well), (2) inclusion of smaller particulate material having a lower intrinsic DDT residue concentration, or (3) exclusion from the centrifuge of larger zooplankters which would be trapped by the phytoplankton net.

Cause 1 represents one reasonable source of loss of DDT residues from the particulate material, if in fact they should have higher DDT residue concentrations than those reported herein. However, experiments with the same cen-

trifuge showed that at least 98% of the particulate chlorophyll *a* in the incurrent water is recoverable from the centrifugal pellet in whole particulate form (trappable on GFC filters). This indicates that breakage of cells must be minimal.

Cause 2 is also a possible explanation. Pfister, Dugan, and Frea (1969) pointed out that chlorinated hydrocarbons showed quantitative differences of distribution among particles greater than 0.15 μ which were separable by density gradient centrifugation. Although they found no recurrent patterns of distribution among the DDT metabolites they were able to detect, their results suggest large differences in the pesticide concentrations in the four different density classes of particles analyzed. The form in which their data are presented, however, does not allow any conclusions about lower or higher DDT residue concentrations in the material which was collected in the centrifuge, but not included in the net-tow material.

Odum, Woodwell, and Wurster (1969) found lower DDT residue concentrations associated with smaller detrital particles in a core taken from a sprayed marsh, but it is uncertain if these results may be applied to oceanic seston.

Cause 3 is a possible explanation on the basis of the mesh size of the zooplankton exclusion filter used in the centrifugation/filtration procedure (0.176 mm) compared to the one used in the processing of the net-tow material both in this report and the earlier published data (0.33 mm).

EFFECT OF STANDING CROP DENSITY

The effect of standing crop density, alluded to above, was observed in the analyses of the particulate material. Standing crop densities were calculated for the transects using estimates of the volume of water filtered during the centrifuge running time and the carbon analyses of the centrifugal pellet. The values for DDT residue concentration are plotted vs. the standing crop density in Figure 3. The slope of the regression line fitted to the data points from both cruises is approximately —1, indicating that equal amounts of DDT residues were taken up by the algal materials within a given volume of water over the range of standing crop densities encountered. This is essentially the same conclusion mentioned earlier (Cox, 1970a).

PARTICULATE MATERIAL AS A PART OF WHOLE SEAWATER

Data points from the Vancouver to San Francisco cruise seem to fit the empirical linear relationship detailed in Figure 3 much more closely ($r = -0.99$) than the data points from the Monterey Bay to southern California cruise ($r = -0.54$). This variability is undoubtedly due to the greater variability of the DDT residue concentrations of the whole seawater from the southern California region, where most of the samples were taken. There would be no need to impute causal relationships between the whole seawater concentration and the concentration of DDT residues in the particulate material, if the particulate material represented a major portion

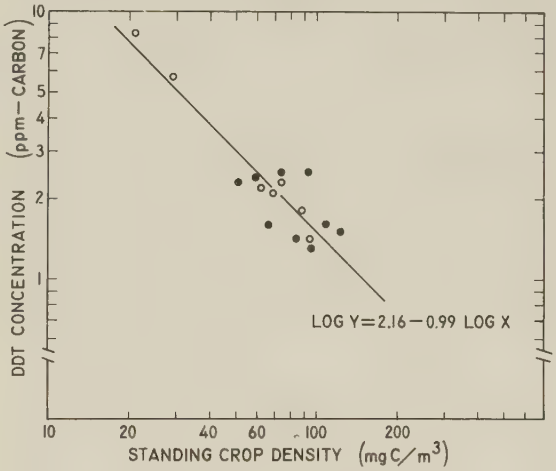

FIGURE 3.—DDT concentrations in the particulate samples as a function of particulate carbon standing crop density. Stations 1 and 22 (Table 4) are not included, since the density of the standing crops could not be computed because there were no measurements of the volume of water filtered in these net-tow samples. Also, for reasons outlined in the text, they may not be comparable to the samples collected by the centrifuge. Transect station 10-11 (Table 4) was omitted because of the possible interference of oil, as described in the text. The remaining 16 values from Tables 3 and 4 appear in this figure. Open circles refer to data from Table 3; solid circles refer to data from Table 4.

of the DDT residues in whole seawater. In fact, the particulate material accounted for less than 10% of the DDT residues in the corresponding whole water extracts (range: 1.8% to 9.9%). Unless the remaining amount of DDT residues (<90% of the total present) is in soluble form, it must be fixed to particles not collected in the centrifugation/filtration procedure. Typical natural distributions of particulate matter in seawater (Bader, 1970; Beardsley, Pak, and Carder, 1970) suggest that most of the particulate volume and almost all of the particulate surface area is accounted for by particles of less than 2 μ in diameter. Thus it is quite likely that the balance of the DDT residues in whole seawater are fixed to these smaller particles, in view of the hydrophobicity and affinity for interfaces characteristic of the different metabolites of DDT. The possibility also exists that it may occur as micelles or aggregates which cannot be taken up by the particulate matter.

EXPERIMENTAL EVIDENCE

Two experiments were performed to examine the distribution of DDT residues between seawater and phytoplankton. In both experiments, ^{14}C-DDT in a 1-ml ethanol carrier was added to GFC filtered oceanic seawater in a 4-liter glass carboy which was stirred by a magnetic stirrer. Repeated subsamples of 25 ml each were taken from the system until successive samples gave a constant ^{14}C activity. All counts were made on a Nuclear-Chicago Unilux II scintillation counter.

Aliquots of a dense suspension of *Dunaliella salina* culture were added to the carboy from a large separatory funnel with a 25-ml dispensing chamber, via a tube connected to the carboy. Sampled and added amounts were such that a constant volume was maintained. After addition of an aliquot of culture, one or two aliquots of 25 ml each were removed from a tap at the bottom of the carboy. This amount was vacuum filtered onto a GFC-glass-fiber filter paper, and counts of ^{14}C-DDT were made of the filter and of a petroleum ether extract of the filtrate. Cumulative ^{14}C activity in the filter and filtrate equalled amounts present in the 25 ml aliquots (both filter and filtrate) before addition of the algal suspension, when the net amounts of ^{14}C-DDT removed from the system by sampling were taken into account. A correction was made for adsorption or possible trapping of small particles of ^{14}C-DDT on the filter. The correction factor, expressed as percent of total activity per 25-ml aliquot which was on the filter before addition of the algal suspension, was constant in the five replicates taken just before the algal cells were added. This correction factor may have changed during the course of addition of the algal cells, but the techniques used did not allow a distinction between ^{14}C activity on the filter which adsorbed, associated with trapped small particles, or associated with the algal cells themselves. I believe that this change was small and did not materially affect the outcome of the experiments.

Figure 4 shows the results of the two experiments. In Experiment 1, the seawater used in the carboy was not altered; in Experiment 2, the seawater was specially prepared to increase the load of small (<1-2 μ) inorganic particles, to see what effect this might have on the uptake function. Nuchar-attaclay, a mixture of finely divided charcoal and clay particles (attapulgite), was added to 2 liters of GFC-filtered seawater. After shaking, the mixture was refiltered through a GFC filter. It is estimated that only a tiny fraction of the initially added Nuchar-attaclay (initially added amount was 0.1 g) actually got through the filter. The 2 liters of water produced in this way were mixed with another 2 liters of GFC-filtered seawater and put into the carboy. Two other conditions were different in Experiment 2. The culture of *Dunaliella salina* used was denser (note that the arrow in Figure 4 indicates that 750 μg C/liter is reached at a lower volume of culture added). The initial concentration of ^{14}C-DDT in Experiment 2 was approximately 15 ppt.

The first part of the uptake functions in Experiments 1 and 2 appeared to be linear, indicating that under the conditions prevailing at the beginning of each experiment, each *Dunaliella* cell took up a constant amount of the ^{14}C-

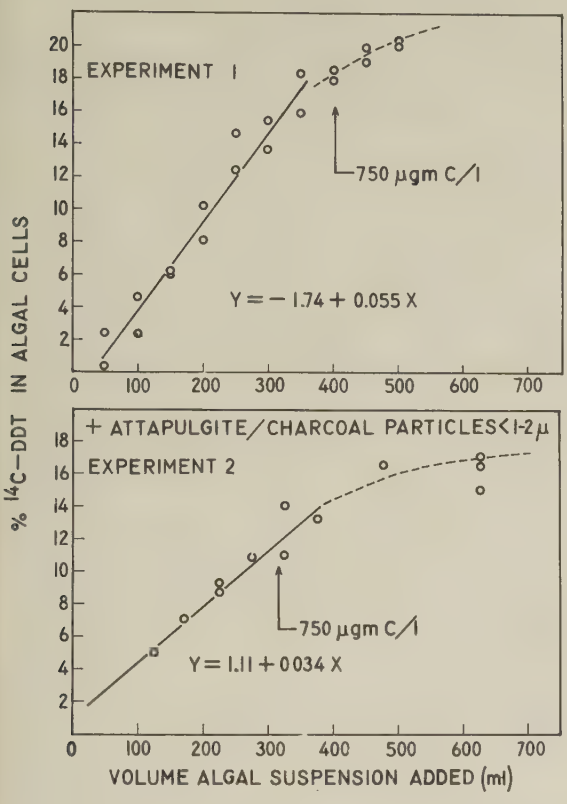
EXPERIMENT 1
750 µgm C/l
Y = − 1.74 + 0.055 X
+ ATTAPULGITE/CHARCOAL PARTICLES<1-2µ
EXPERIMENT 2
750 µgm C/l
Y = 1.11 + 0034 X
% 14C-DDT IN ALGAL CELLS
VOLUME ALGAL SUSPENSION ADDED (ml)

FIGURE 4.—Percentage of total ^{14}C-DDT in sample aliquots recovered on GFC filters, plotted as a function of total volume of *Dunaliella salina* culture added to a constant volume system. See text for detailed discussion.

DDT which was available. Both curves show inflection points after the density of cells increases beyond 750 µg C/1. The fact that the uptake per cell was constant over the linear range indicates that each cell has a saturation value for uptake of DDT, which is independent of the ambient concentration of DDT available. The curves presumably begin to level off when the ^{14}C-DDT which was available for uptake is mostly associated with the algal mass already added.

If algal cells exhibit a saturation value for uptake of DDT, then adsorption of DDT to the cell surface is a more likely explanation for DDT uptake than phase partitioning of DDT between seawater and the lipid component of the algal cell, as has been previously hypothesized (Cox, 1970b). Each *Dunaliella salina* cell probably had a total cell surface area of 240 μ^2 (Mullin, Sloan, and Eppley, 1966). The cells in Experiments 1 and 2 took up a mean of 5×10^{-5} picograms ^{14}C-DDT/μ^2. This value may be near the asymptotic saturation value for *Dunaliella salina* for the experimental conditions described above. The validity of a saturation value of this kind needs to be tested with other phytoplankton species over a wide range of ambient DDT concentrations.

A quantitative solution to simultaneous Freundlich adsorption equations for the algal cells and the <1-2 µ particles could explain the uptake curves if the adsorption energy coefficient were known in each case. Studies such as those of Weber and Gould (1966) should therefore be applied to uptake of DDT residues by phytoplankton and smaller particles to elucidate the relationships discussed here.

No measurements were made of the concentration of the <1-2 µ particles in the untreated seawater of Experiment 1 or the treated seawater of Experiment 2. Thus the differences can only be explained qualitatively. The higher concentration of ^{14}C-DDT in Experiment 2 (30 ppt) was apparently reflected in the uptake of ^{14}C-DDT per unit of cell surface area; Experiment 2 yielded a value of about 6×10^{-5} picograms ^{14}C-DDT/μ^2, which is higher than the mean for both experiments quoted above. The uptake of ^{14}C-DDT per unit of cell surface area in the case of Experiment 2 is probably closer to the asymptotic saturation value because of the higher concentration of ^{14}C-DDT in the medium. The main difference between the curves for Experiments 1 and 2 is the position of the inflection point. Experiment 2 shows an apparent inflection point which is lower than the apparent inflection point of Experiment 1, indicating a lowering of the available percentage of total ^{14}C-DDT in the system. The total small particle concentration of the system was not measured, so this apparent change must be regarded as a presumptive effect of the Nuchar-attaclay addition.

If a large percentage of the DDT added to aqueous systems is fixed to a particle fraction

less than 1-2 μ in diameter, then the experimental DDT uptake results that have been interpreted in terms of a partition coefficient or a concentration factor using the nominal concentration of DDT in the aqueous medium become relatively meaningless without knowledge of the fraction of the initial amount of DDT present which is fixed to these small particles and hence unavailable to the test organism. In the oceanic environment, it is quite likely that the amounts of "available" DDT residues are exceedingly small. Uptake of DDT by the seston is probably closely coupled with input pulses which would be largely determined by fallout conditions at the surface and seasonal runoff of DDT residues from land areas. "Available" DDT residues which may rise during these periods will be taken up by plankton. A complete picture of the processes involved in DDT transport to the pelagic environment has yet to be drawn and will require further experimental and analytical work.

ACKNOWLEDGMENTS

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EGG LOSS DURING INCUBATION FROM OFFSHORE NORTHERN LOBSTERS (DECAPODA: HOMARIDAE)

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ABSTRACT

Egg loss during incubation from offshore northern lobsters, *Homarus americanus* Milne Edwards, was estimated by counting the eggs of 196 females. The lobsters were captured along the continental shelf off southern New England during October (eggs recently extruded), April, and June (eggs nearly ready to hatch). Egg loss during the period October to June averaged 36% for females of all sizes studied.

The exploitation of northern lobsters in the offshore canyons of the continental shelf is steadily increasing (Skud, 1969). Owing to this increased effort, the National Marine Fisheries Service (formerly the Bureau of Commercial Fisheries) has initiated a study of the biology and population dynamics of the stock. As accurate estimates of fecundity are useful for studying population dynamics, the present study was undertaken to determine the extent of egg loss from female *Homarus americanus* during embryonic development and the consequential magnitude of error in estimating fecundity if any loss occurs. Brunel (1962, 1963) has shown that female spider crabs, *Chionoectes opilio*, may lose over half their eggs during the incubation period. The present paper shows the extent of egg loss from the northern lobster and the difference in fecundity estimates depending on the time during the development period when the eggs are counted.

METHODS AND MATERIALS

The lobsters were captured with otter trawls during research and commercial cruises at Hudson, Veatch, Oceanographer, Lydonia, and Corsair Canyons. These canyons are located along the edge of the continental shelf, south and east of New England. The 196 female lobsters used in this study were divided into 5-mm groups according to carapace length. The mean number of eggs and the range for each 5-mm group are recorded in Table 1. Carapace length was measured from the posterior edge of an eye socket to the distal edge of the carapace.

The females were frozen at sea, later thawed in the laboratory and their eggs removed from the pleopods by stripping with small forceps. The eggs were hardened in Formalin for 24 hr, then soaked in fresh water before being dried in an oven at 150° C. Drying time lasted 1 to 2 hr depending on the size of the egg mass. After drying, the individual egg masses were rubbed over a 1-mm screen to break up any clusters and eliminate non-egg material, then counted with an electronic counter (Boyar and Clifford, 1967). Test runs with the counter produced a maximum error of $\pm$ 2%. The counts given represent numbers of viable eggs only (nonviable eggs are rarely observed in masses of developing eggs).

RESULTS AND DISCUSSION

A curvilinear relationship was apparent when the number of eggs was plotted on the corresponding carapace length. The same result was noted by Saila, Flowers, and Hughes (1969). In order to employ covariance analyses in this study, the data were transformed to natural logarithms. The lines presented in Figure 1 were plotted from the antilogarithms of the values calculated from the linear regression equations (Table 2).

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TABLE 1.—Month of capture, range and mean number of eggs for each 5-mm carapace length group for the 196 offshore female lobsters.

Carapace length groups (mm)	OCTOBER			APRIL			JUNE		
	Number of lobsters	Number of eggs		Number of lobsters	Number of eggs		Number of lobsters	Number of eggs	
		Mean	Range		Mean	Range		Mean	Range
80-84	3	10,449	8,286-11,357	1	9,212	--	1	7,890	--
85-89	10	14,341	8,707-17,428	2	10,518	9,010-12,027	2	7,920	6,400- 9,440
90-94	23	16,317	11,501-21,348	4	13,476	9,578-18,400	5	8,950	6,410- 12,240
95-99	15	19,440	14,425-25,454	3	15,973	12,140-19,310	3	9,887	8,270- 10,700
100-104	15	20,463	13,831-26,832	8	19,355	12,853-23,270	6	14,900	8,470- 17,940
105-109	1	24,896	--	4	19,212	16,161-22,853	4	19,692	10,160- 30,190
110-114	3	30,452	27,321-32,309	11	23,789	17,639-28,245	0	--	--
115-119	0	--	--	7	27,001	18,703-33,160	1	30,602	--
120-124	2	44,334	42,059-46,610	1	26,628	--	3	27,743	20,671- 31,789
125-129	2	51,184	50,008-52,361	5	35,757	18,821-40,159	1	42,743	--
130-134	0	--	--	5	32,796	25,180-41,636	4	40,728	33,238- 49,820
135-139	1	55,240	--	2	31,045	26,850-35,240	4	40,092	22,600- 52,956
140-144	0	--	--	2	51,002	48,526-53,478	4	45,506	32,770- 54,820
145-149	0	--	--	1	45,928	--	2	68,510	67,660- 69,360
150-154	0	--	--	0	--	--	5	52,457	44,463- 69,095
155-159	0	--	--	1	78,422	--	3	51,978	37,400- 66,551
160-164	0	--	--	1	66,210	--	3	64,673	56,302- 73,207
165-169	0	--	--	0	--	--	2	63,175	62,573- 63,777
170-174	0	--	--	1	70,178	--	3	71,831	60,866- 87,303
175-179	0	--	--	0	--	--	1	81,164	--
180-184	0	--	--	0	--	--	3	73,770	56,372-104,541
185-189	0	--	--	0	--	--	0	--	--
190-194	0	--	--	0	--	--	1	78,844	--
195-199	0	--	--	0	--	--	1	90,400	--

TABLE 2.—Raw sums, sums of squares, sums of cross products, and regression equations derived from transformed data on carapace lengths and numbers of eggs from 196 berried female lobsters captured during October, April, and June. ($X = \log_e$ carapace length, $Y = \log_e$ number of eggs.)

Month	N	ΣX	ΣX^2	ΣY	ΣY^2	ΣXY	Regression equation ($Y = a+bX$)
October	75	342.8625	1568.1306	737.7546	7267.0013	3375.0329	$\hat{Y} = -5.0202+3.2499(X)$
April	59	279.4272	1324.7648	595.6230	6026.4485	2824.7918	$\hat{Y} = -3.2280+2.8132(X)$
June	62	301.6997	1471.6059	640.9857	6666.0539	3130.1578	$\hat{Y} = -5.0231+3.1569(X)$

The regression lines were all significantly different ($P < 0.01$) in level (Y intercept) from each other. No significant difference was found in the slope of any of the lines, indicating egg loss is consistent between extrusion and hatching from females of all sizes. The average total loss from extrusion to hatching throughout the size range of the females studied was 36%, and the average loss during the last few months (April through June) was 13%.

The eggs obtained in October were all in prenaupliar condition (Templeman, 1940) and were judged to be no more than 4 weeks in age. Those eggs obtained in June were all within a month of hatching. Twenty-four berried females, caught in June, were kept in laboratory tanks where all egg hatching was completed by the middle of July. Eggs taken in April showed less development than those taken in June, and 12 females kept in the laboratory tanks from the April samples completed hatching by the first of July. Water temperatures in the laboratory during April and May were slightly higher than would be expected in the offshore waters for the same period.

The offshore waters where the lobsters are found undergo no great seasonal temperature fluctuations (Colton et al., 1968) typical of the coastal waters of New England. I have examined over 500 berried females from the offshore area and conclude that extrusion of eggs occurs in September and October, and that hatching occurs the following June or July. I have found no deviation from this pattern; however, slight

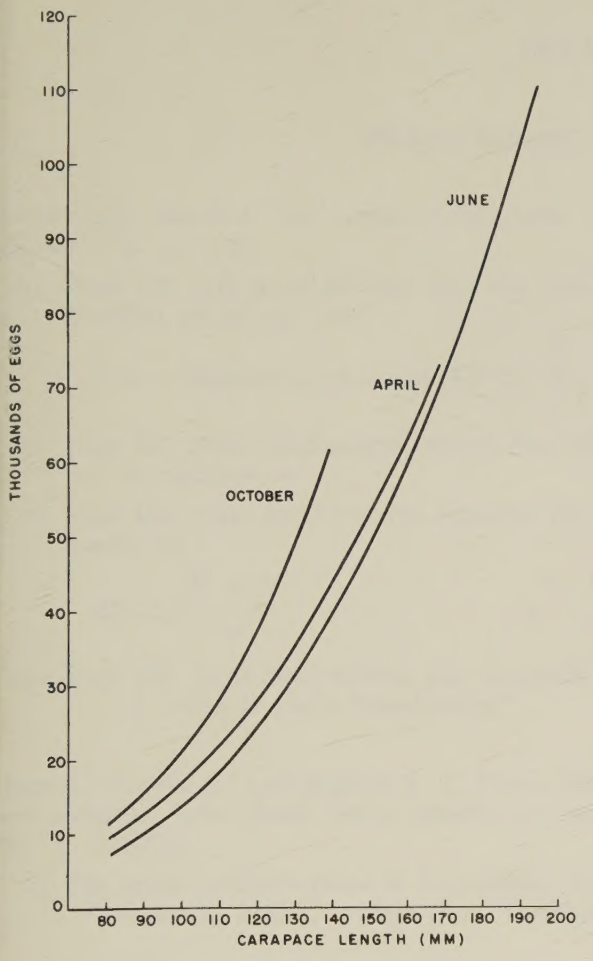

FIGURE 1.—Regression lines of egg counts versus carapace lengths for October, April, and June.

variation in the extent of development of eggs from one year to the next during the same month has been noted, and from canyon to canyon during the same year. These variations among years and areas are attributed to small differences in water temperature, while the consistent year to year reproductive periods are attributed to the relatively constant thermal environment.

While counts obtained from ovarian or newly extruded eggs testify to the reproductive potential of a female, counting eggs that are close to hatching give more reliable estimates of a female's potential contribution of larvae to the population.

ACKNOWLEDGMENTS

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ERRATA

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ROTHSCHILD, BRIAN J., AND JAMES W. BALSIGER, "A linear-programming solution to salmon management," p. 117-140.

1) Page 120, left hand column, first line below equation (4) has the inequality in the wrong direction and should have:

"The constraint is redundant if $K' \geqslant \sum_{j=1}^{N} K_j$."

2) Page 120, right hand column, fourth line below equation (8) should have same script "$\mathcal{M}$" as "$\mathcal{M}$" in equation 8.

3) Page 120, right hand column, equation (9) has the inequality in the wrong direction and should be:

$$\Sigma W_{ij} \geqslant \frac{E \times H}{F}$$

4) Page 137, right hand column, line 40 should read:
... a series of years "maximizing" ...

SECKEL, GUNTER R., AND MARIAN Y. Y. YONG, "Harmonic functions for sea-surface temperatures and salinities, Koko Head, Oahu, 1956-69, and sea-surface temperatures, Christmas Island, 1954-69," p. 181-214.

1) The senior author's name is misspelled. It should be:
"Gunter R. Seckel," rather than "Gunther R. Seckel."